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(54) **Humanized monoclonal antibodies.**

(57) A method of preparing humanized monoclonal antibodies is described which utilizes comparative model burding methodology. A humanized anti-CD18 antibody, 60.3, has been formulated and demonstrated to have analogous binding characteristics to the original murine monoclonal antibody, while displaying essentially complete human Ig heavy and light chains.

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Technical Field

Th present invention is directed to a method for producing humanized monoclonal antibodies by utilizing comparative model building to construct the humanized antibody from homologous regions of human proteins by rational design. Specific humanized monoclonal antibodies are prepared.

Background of the Invention

Murine derived monoclonal antibodies have been utilized as diagnostic and therapeutic agents for numerous human pathologic conditions including acute inflammatory responses associated with numerous diseases. Administration of murine derived monoclonal antibodies (mAbs) as therapeutic agents in man has been severely limited by the development of antibody within the recipient to the mouse antigens of the murine derived monoclonal antibody. In attempts to circumvent this outcome mAbs have been restructured by recombinant DNA technology in such a way as to decrease their immunogenicity in humans. Immunoglobulins are well defined both chemically and biologically with the general structures illustrated in Molecular Cell Biology. Darnell, Lodish and Baltimore, Eds. Scientific American Book, Inc. W.H. Freeman, New York, NY (1986). Initially, this involved the construction of chimeric antibodies, Morrison *et al.* Proc. Natl. Acad. Sci. USA 81: 6851-6855 (1984). Recombinant technology was employed to replace the murine heavy and light chain constant regions with corresponding human constant regions. Upon expression, such interspecies antibody chimeras yielded molecules with the antigen binding specificities of the parent murine antibody. The following references generally describe chimeric antibody technology: Lobuglio *et al.* Proc. Natl. Acad. Sci. USA 86: 4220-4224 (1989); United States Patent 4,816,567; PCT International Publication No. WO 87/02671, published May 7, 1987; European Patent Publication No. 255,694, published February 10, 1988; European Patent Publication No. 274,394, published July 13, 1988; European Patent Publication No. 323,806, published July 12, 1989; PCT International Publication No. WO/89/00999, published February 9, 1989; European Patent Publication No. 328,404, published August 16, 1989; European Patent Publication No. 322,424 published September 13, 1989, and European Patent Publication No. 438,310 published July 24, 1991.

The immunogenicity of chimeric antibodies can be further reduced by grafting rodent hypervariable regions into the variable region frameworks of human light and heavy chains, Jones *et al.* Nature 321: 522-525 (1986). These hypervariable regions have also been termed complementarity determining regions (CDR). The technique involves the substitution or recombinant grafting of antigen-specific murine CDR sequences for those existent within "generic" human heavy and light chain variable regions, European Patent Publication No. 239,400, published September 30, 1987. In this approach, little, if any, concern is shown for the variable region frameworks (FRs) within which the murine CDR's are placed.

Studies by Queen *et al.* Proc. Natl. Acad. Sci. USA 86: 10029-10022 (1989), have shown the CDRs from a murine anti-Tac monoclonal antibody can be grafted into a human framework. The human framework variable regions were chosen to maximize identity with the murine sequence. The authors also utilized a computer model of the mAb to identify several amino acids which, while outside the CDRs, are close enough to interact with the CDRs or antigen. These residues were mutated to the residue found in the murine sequence. The grafted anti-Tac antibody had an affinity for the antigen which was only about 1/3 that of the murine anti-Tac mAb.

Leukocyte infiltration into an inflammatory site is dependent on the adhesion of the leukocytes to the endothelium prior to extravasation. The rapid binding of polymorphonuclear leukocytes (PMN) to the endothelium and diapedesis occurs within minutes after the introduction of a chemotactic stimulus in tissue, Cybulski *et al.*, Am. J. Pathol. 124: 367 (1986). This rapid extravasation appears to depend on the response of the PMNs to chemoattractants and on the presence of the CD11/CD18 family of glycoproteins on the leukocyte surface. The family of glycoproteins associated with PMNs are termed leukocyte integrins and include LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18) and p150,95 (CD11c/CD18). Each of these heterodimers has a unique alpha chain (CD11 a, b, c) and an invariant beta-2 chain (CD18). Stimulation of PMNs with various chemotactic factors causes increased expression of leukocyte integrins (CD11b/CD18) fostering strong adhesion to unstimulated endothelium *in vitro*; Harian, Blood 65: 513 (1985). Essentially all of the chemoattractant-induced adhesion is inhibited by treating the PMNs with mAbs specifically reactive with the CD11/CD18 complex, Harian *et al.*, Blood 66: 167 (1985); Zimmerman and McIntyre J. Clin. Invest 81: 531 (1988); Smith *et al.*, J. Clin. Invest 82: 1746 (1988) and Lo *et al.*, J. Exp. Med. 169: 1779 (1989).

Murine hybridomas producing monoclonal antibodies reactive with the beta chain common to the Mac-1, LFA-1 and the p150,95 integrins have been described. The mAbs are designated 1B4, 60.3, TS1/18, H52 and ATCC TIB 218. The 1B4 is an IgG1 antibody and was prepared by Wright *et al.* Proc. Natl. Acad. Sci. USA 80: 5699-5703 (1983), the 60.3 is also IgG2a and was prepared by Beatty *et al.* J. Immunol. 131: 2913-2918

(1983), TS1/18 is an IgG1 antibody and was prepared by Sanchez-Madrid *et al.*, J. Exp. Med. 158 : 1785-1803 (1983), and ATCC TIB 218, a IgG2a kappa prepared by Springer *et al.*, J. Exp. Med. 158 : 586-602 (1983). These antibodies appear to be functionally equivalent and cross-react with the beta 2 - chain found in human, sheep, pig, rabbit and dog leukocytes but not with the beta-2 chain found in murine and rat leukocytes.

## Summary of the Invention

The present invention is directed to a method for producing humanized monoclonal antibodies by utilizing a process of comparative model building. In this method computer data bases are searched to locate homologous human protein sequences that correspond to specified regions of the non-human derived (usually murine) antibody, and a series of models is formulated, tested and modified to produce a model of a humanized antibody which is then constructed by recombinant DNA technology. In a preferred embodiment, a humanized monoclonal antibody corresponding to the murine anti-CD18 antibody 60.3 was prepared.

The variable (V) region sequences from both the heavy (H) and light (L) chains were determined from cDNA (amplified by PCR), and spliced onto human constant (C) regions, resulting in a chimeric 60.3 Ab (IgG1, kappa). The chimeric Ab was expressed in tissue culture (Ag8.653 mouse myeloma cells, detected by ELISA), and examined in binding assays. The results from competition and inhibition assays showed that the chimeric Ab was as effective as the murine 60.3 mAb.

The deduced murine  $V_H$  and  $V_L$  protein sequences were compared to the protein sequence data base, and two human Ig protein sequences were selected to be used as templates. The present inventors modeled a murine 60.3 Fv according to the deduced  $V_H$  and  $V_L$  protein sequences. Based on the 60.3 Fv model and the two human template sequences selected from the protein data base, a humanized Fv was modeled.

Construction of the humanized 60.3 was done by piecing 5 pairs of complementary oligonucleotides together (spanning the entire V region) to form the  $V_H$  and  $V_L$ . These were then attached onto vectors containing genes for appropriate C regions to form humanized Ab (IgG1, kappa). The humanized proteins were again expressed in Ag8.653 cells and binding assays were done. FACS analyses indicated that the humanized Ab recognized cells expressing CD18. About a dozen of the humanized 60.3 Ab master wells were transferred and assayed for Ig.

## Brief Description of the Drawings

In the drawings:

Figure 1 illustrates an amino acid comparison of the murine 60.3 antibody heavy chain (m60.3) with the human variable heavy chain consensus sequence for the framework regions of human subgroup  $V_{H1}$  (hVh1/Jh4), the human template (M030) used for humanization (h60.3 template), a germline sequence homologous to M030 (21-2'CL), and phases I to IV of the humanization process. All amino acids which are identical to the phase IV sequence are shaded. In addition, sequences which are different than the previous phase sequence are shown in bold.

Figure 2 illustrates an amino acid residue comparison of the murine 60.3 antibody light chain (m60.3) with the human variable light chain consensus sequence for the framework regions of human subgroup  $V_{K1}$  (hVkl/Jk), phases I-IV of the humanization process and the human template used for the humanization (h60.3 template). All amino acids which are identical to the phase IV sequence are shaded. In addition, sequences which are different than the previous phase sequence are shown in bold.

FIGURE 3 illustrates five pairs of complementary oligonucleotides corresponding to the variable regions of the light chain.

FIGURE 4 illustrates five pairs of complementary oligonucleotides corresponding to the variable region of the heavy chain.

FIGURE 5 illustrates the binding of murine, chimeric and humanized 60.3 antibody to HL60 human myelomonocytic cells. Fluorescein isothiocyanate (FITC) labelled antibody was incubated with cells at the concentrations indicated on the abscissa and the amount of antibody bound is indicated by relative fluorescence intensity on the ordinate.

FIGURE 6 illustrates the competition by preincubation of cells with chimeric and humanized 60.3 of the binding of FITC-labelled murine 60.3 to HL60 cells. HL60 cells were preincubated with 1 ug/ml of either chimeric 60.3 (circles) or humanized 60.3 (squares), followed by incubation with various concentrations of FITC-labelled murine 60.3. In the absence of competing antibody, FITC - m60.3 binding to the HL60 cells increased with increasing concentration (x).

FIGURE 7 illustrates the direct competition of FITC-murine 60.3 binding to HL60 cells by chimeric and humanized 60.3. The dashed line shows the fluorescent intensity of binding by FITC-murine 60.3 in the absence

of competitor, while additions of increasing concentrations of chimeric 60.3 (squares) and humanized 60.3 (circles) inhibited FITC-m60.3 binding.

FIGURE 8 illustrates the results of a chemiluminescence binding assay of murin (closed square), chimeric (open square) and humanized (closed diamond) 60.3 antibody upon HL60 cells. The anticancer antibody L6 (open diamond), which does not bind to HL60 cells, was used as a control.

FIGURE 9 illustrates a series of restriction maps for plasmids utilized in the production of the variable light chain plasmid pGK.11.

FIGURE 10 illustrates the nucleotide sequence for the humanized variable light chain.

FIGURE 11 illustrates a series of restriction maps for plasmids utilized in the production of the variable heavy chain plasmid pNy1.16.

FIGURE 12 illustrates the nucleotide sequence for the humanized variable heavy chain.

#### Description of Preferred Embodiments

The present invention is directed to a method of producing humanized monoclonal antibodies (mAbs) by utilizing a process of comparative model building and rational design. In a preferred embodiment this method is utilized to produce a humanized molecule of the anti-CD18 murine monoclonal antibody 60.3. The mouse mAb 60.3 (IgG2a), which recognizes a functional epitope on the beta subunit (CD18) of leukocyte integrins, prevents the adherence and aggregation of polymorphonuclear neutrophils (PMN), resulting in a blockage of neutrophil mediated damage during shock and reperfusion injury. It would be advantageous to modify mAb 60.3 to reduce the potential for HAMA (human anti-mouse Abs) response. Therefore, in one embodiment, the present invention was directed to "humanize" the 60.3 mAb by creating an Ab whose constant (C) region is human, but whose variable (V) region is composed of both human (principally framework sequences) and mouse (principally CDR loops) sequences. For the studies described in the present invention, murine, chimeric and humanized antibodies were purified from solution by protein A chromatography on IPA - 400 Fast Flow Immobilized rProteinA (Repligen, Cambridge, MA) using the manufacturer's recommended protocol.

In the present invention recombinant methods are utilized to produce humanized monoclonal antibodies that contain complementarity determining regions (CDRs) analogous to the originally derived monoclonal antibody, and which have homologous human heavy and light chain framework regions. The resulting antibodies demonstrate the binding affinity and specificity of the original antibody yet are completely humanized monoclonal antibodies.

As used herein the term "humanized" and its various grammatical forms as it relates to antibodies is defined to mean that the amino acid residues of the antibody in the heavy and light chains are replaced with amino acid residues corresponding to homologous human protein regions without altering the binding activity of the antibody. For the humanized 60.3 monoclonal antibody of the present invention there is approximately 80% sequence identity of the variable regions of the heavy and light chains with those of the human mAb, while the constant regions are distinctly human. Some variation of individual amino acids in the antigen binding and framework regions are contemplated by this invention and are within the scope of this invention when such variations do not interfere or inhibit the binding to antigen, such as the Ile for Glu substitution at position 106 of the light chain.

As used herein the term "canonical loop conformation" refers to a small repertoire of main chain conformations for five of the six loops (all except H3). The particular conformation adopted is determined by only a limited number of residues within the loop or the framework.

As used herein the term "framework residues" means residues which are located outside the structurally defined CDR loops. These residues can be part of the hypervariable regions for the antibody.

As used herein the term "monoclonal antibody" refers to all recombinant antibodies derived from an initial single cell and includes murine monoclonal antibodies, chimeric antibodies and humanized antibodies.

In the present invention a procedure of comparative model building was utilized to construct the appropriately designed humanized antibody. As a preferred embodiment, the modeling of the murine 60.3 antibody is summarized.

The Brookhaven Protein Database (Bernstein et al. (1977) J. Mol. Biol. 122 : 535-542) was searched for the antibody crystal structures which show reasonably high homology (> 50% sequence identity) to the variable regions of murine 60.3. If the variable light chain and heavy chain templates which fulfill these criteria are from different antibodies, these structures are combined by superposition of the set of structural invariants at the V<sub>L</sub>-V<sub>H</sub> domain interface (Nivvotny et al. (1985) Proc. Natl. Acad. Sci. USA 82 : 4592-4596). This provides the "structural template" for model building of murine 60.3 (and humanized 60.3 below).

The CDR loops and their known structural framework determinants of murine 60.3 are determined by defining the CDR loops structurally according to the method of Chothia et al. (Chothia et al. (1989) Nature 342 :

877- 883). The structurally defined CDR loops consist on average of shorter sequence segments than the hypervariable regions defined by Kabat (Kabat et al (1987) Sequences of Proteins of Immunological Interest). The five canonical CDR loops (L1-13, H1-H2, i.e. all except H3) in the 60.3 variable light and heavy chain are assigned to known canonical loop conformations, and the framework residues which are crucial for the conformation of the CDR loops are determined.

The non-canonical H3 loop region within the 60.3 sequence is defined and a model of murine 60.3 is then built. The CDR loops of the structural template are replaced with canonical CDR backbone templates as determined using interactive computer graphics (INSIGHT II, Ver. 2.0 Biosym Technologies, Inc. 1991). Loop searches (Jones et al (1986) *Embo J.* : 819-822) in the Brookhaven Protein Data Bank are carried out to extract an initial backbone approximation for the non-canonical CDR loop H3.

All non-conserved amino acid side chains in similar positions are replaced using interactive computer graphics. The model then consists of a combination of backbone fragments of different antibodies with replaced side chains. The model is solvated with a 7 Å water layer and the structure is refined using an energy minimization (Mackay et al. (1989) *Prediction of Protein Structure and the Principles of Protein Conformation*. New York: Plenum Press pp.317-358) protocol where, over the course of 1600 cycles of conjugate gradients minimization, constraints of 80 kcal/mol/Å<sup>2</sup> on all protein non-hydrogen atoms are gradually released until, at the final stage of the minimization procedure, all atoms of the system are free to move.

The most homologous human variable region sequences are found by searching the sequence data base for the most homologous human sequences for the variable light and the variable heavy chains of the 60.3 antibody and these sequences are combined to obtain a "human template". The structural template for murine 60.3 is confirmed to be suitable for the human template. The sequences of the structural template chosen initially showed > 50% sequence identity to the variable regions of the "human template". Furthermore the percent homology is chosen to be similar to that found for comparison of the structural template with the murine sequence. The CDR loops and known structural determinants are then grafted onto the human template (Jones et al. (1986) *Nature* 321 : 522- 535). The CDR loop regions and structural determinants in the "human template" sequence are replaced by the analogous sequences from the murine antibody, as determined above. This provides the Phase I h60.3 sequence. A Phase I model of humanized 60.3 is built using the same model building protocol as described for murine 60.3.

In Phase II the murine and Phase I h60.3 models were compared. These models now consist of the murine binding site and murine framework (murine 60.3 or m60.3) and of murine binding site and human framework (Phase I humanized 60.3 or h60.3). The murine and Phase I h60.3 were superimposed using the structural invariants of the immunoglobulin fold (Novotny et al. (1985) *Natl. Acad. Sci. USA* 82 : 4592-4596). The models of the binding site regions were compared residue by residue from the N-terminus to the C-terminus. By this comparison, all framework residues and residues within the framework - CDR junctions which can interact with the murine CDR loops and may therefore be important for the structural integrity of the murine binding site were identified. These residues typically include all the known structural determinants for the specified canonical CDR loop conformations (Chothia et al. (1989) *Nature* 342 : 877-883) and other residues found to be critical in the comparison (due to proximity to the CDR loops and potential for interaction with them). These residues were then "re-mutated" to the murine residues, forming the Phase II h60.3 model.

The murine 60.3 model and the modified humanized sequence were then further refined by again subjecting the models to the energy minimization procedure described above. This construct represents the Phase II h60.3 model.

In Phase III, further improvements of the structural model of h60.3 were made. A conformational search (Brucoleri RE and Karplus M. (1987) *Biopolymers* 26 : 137- 168) was carried out over regions of the binding site which cannot be directly assigned to known structural templates. Typically, this is the CDR loop H3 and perhaps one or more CDR loops which may not belong to known canonical structure types. Side chain conformations of the antigen binding site loops and the framework - CDR junctions are also further refined using an iterative conformational search protocol (Brucoleri RE and Karplus M. (1987) *Biopolymers* 26 : 137-168). The refined model structure may be called Phase III h60.3.

In Phase IV, analysis of the binding site features of the Phase III h60.3 model was carried out. The binding site features of the construct were analyzed in detail in order to classify the antibody structure, for example, as a "groove-type" or "cavity-type" or "flat" antibody. This allows one, in the absence of detailed structural knowledge of the antibody-antigen complex, to postulate which parts of the CDR surface or residues at the CDR-framework junctions are unlikely to be involved in antigen binding. In the Phase III and earlier models, these positions may be occupied by murine residues which can now be changed to human residues.

This improves the "degree of humanization" of the antibody since parts of some CDR loops and the entire CDR loops can be "humanized". At this stage, the final version of humanized 60.3, Phase IV h60.3 was obtained.

Comparative molecular modeling has been used here to enable a detailed three-dimensional comparison of a murine antibody and its humanized version. This comparative study has enabled the present inventors to analyze residue-residue interactions which are likely to be critical to retain the murine specificity in the structural context of a largely human antibody. Furthermore, the different modeling concepts based on structural homology (experimental structural data) and conformational search (which represents an *ab initio* method) have been combined to obtain the best possible picture of the 60.3 binding site in order to, gain some insight into which of the binding site residues may be not involved in antigen binding.

In addition to the application summarized above, comparative model building can be applied to other problems. For example, many of the antibody structures which are modeled today are used to guide mutagenesis experiments in order to explore affinity and antigen specificity. Such antibodies are often modeled because experimental structures are not available for these antibodies. Comparative model building provides an opportunity to assess the confidence level of such theoretically derived structures.

For example, the combining site of a clinically relevant antibody can be derived starting from different structural templates and employing the different methods based on structural homology and conformational search. By pairwise combination of two different templates with two different methods, four model structures can be derived in an independent way and then compared by superposition of structural invariants. This comparison allows for the determination of how well the independently derived structures agree and which parts of the models do not show satisfying agreement. In the absence of experimental structural data, such comparative model building exercises presently provide the only way to assess the confidence level of antibody models. If the independently derived structures agree well, a high confidence level can be assigned to the model and a "consensus model" can be prepared. The consensus model would then typically represent a combination of structural elements derived by structural homology and conformational search. On the other hand, disagreement of the models allows for the identification of the particular critical regions in model structures which are less well defined and need to be improved or, if this is not possible, treated with caution. Such knowledge, obtained by comparative model building, is very important for the use of model structure for experimental design.

Although the humanized 60.3 Ab was prepared by grafting the murine CDRs onto the human frameworks, certain amino acids were not changed from the murine protein sequence to their human counterpart (due to their importance in retaining the conformation of the CDR loops). Therefore, three humanized 60.3 L "mutants" (based on computer modeling) were constructed in an effort to 1) further reduce its divergence and 2) determine the contribution of these amino acids on antigen (Ag) binding.

There are 4 amino acids in the L chain which are changed: one is in the CDR2 (postulated not to be involved in binding) and the remaining amino acids all reside in the framework 2 region. The three humanized 60.3 L "mutants" are as follows:

- 1) Mutant 1: amino acid change is only at position 50, from Arg (R) to Asp (D).
- 2) Mutant 2 : 3 amino acids changes are carried out at positions 50 (as in Mutant 1), 54 [from Leu (L) to Arg (R)] and 55 [from Glu (E) to Ala (A)].
- 3) Mutant 3: contains the changes in Mutant 2, and at position 68 Arg (R) is changed to Gly (G).

From this set of mutants, effects can be seen on binding by (1) Arg 68 alone (by comparing results from Mutants 3 and 2) and (2) Leu 54 and Glu 55 (by comparing results from Mutants 2 and 1).

Total cellular RNA was extracted from a 60.3 producing cell-line according to the method of Davis et al (1986). The first strand cDNA was synthesized using a cDNA synthesis kit from InVitrogen and an oligo (dT) primer. The cDNA was then amplified by polymerase chain reaction (PCR) using degenerate primers (Larrick, et al.(1991) Scand. J. Immun. 32 :121-128; Colloma and Larrick (1991) Biotechniques 11 : 152-156). For the heavy chain, the sense primers (MH-SP-ALT.1 and MH-SP-SLT.2) were from the signal peptide and had an Xho I restriction site and the 5' end. The antisense primer (MH- gamma-CONST) was a consensus sequence from the CH1 domain of murine  $\gamma$  heavy chains and had a Pst I site at the 3' end.

For the PCR amplification of the light chain, the sense primers (EcoRI/FR1-ML (k)) were from the 5' end of the first framework region and had an Xho I restriction site at the 5' end. The antisense primer (HindIII/M1(k)-CONST) was from the k constant region, but had a Sal I restriction site instead of the Hind III site described by Larrick et al.

For both the heavy and light chains, the PCR products were either restricted (Xho I and Pst I for the heavy chain; Sal I and Xho I for the light chain) and cloned into similarly restricted pUC 18 or treated with nucleotide kinase followed by blunt ended ligation into Sma I digested pUC 18. Ligation products were used to transform competent DH $\alpha$  E. coli cells.

Clones containing inserts were selected using X-Gal/PTG; positive clones were screened for appropriately sized EcoR I-Sal I restriction fragments. EcoR I and Sal I flank the cloning sites in pUC 18 and are therefore expected to release the PCR product producing an approximately 0.5kb fragment. Selected clones were sub-

jected to the double stranded DNA sequencing (Hsiao (1991) Nucl. Acid Res 19 :2787) using Sequenase (U.S.Biochemical). The sequence is shown in Figures 10 and 12, and Sequence I.D. numbers 9 and 10. The V gene sequences were compared to sequences of other murine Ig genes (Kabat *et al.*, (1987) Sequences of Proteins of Immunological Interest, 4th ed., Nat. Inst. of Health, Bethesda, MD.) The heavy chain was found to belong to the VH IIa subgroup and the light chain to belong to the Vk IIIb subgroup.

In order to be sure that the correct V genes had been cloned and sequenced, heavy and light chain from purified 60.3 were subjected to N-terminal amino acid sequencing. The amino acid sequence of the heavy chain was identical to that deduced from the DNA sequence. However, there was a discrepancy for the 7th and 8th amino acids of the L chain. For the DNA, these amino acids (Tyr and Gln, respectively) are encoded by the sense primer used for the PCR reaction. Ser and Pro were found at these positions by amino acid sequencing. Furthermore, almost all other Vk genes of this subgroup had Ser and Pro at this position. It was concluded that the primers used were not entirely appropriate for the V gene. They were however, similar enough to the cDNA that annealing and priming could occur. The codons for these 4 amino acids occur at the 3' end of the primer and are as follows:

Tyr Gln	:	TA (C/T)	CA (A/G)
Ser Pro	:	TCX	CCX

To determine the real sequence at this position, the entire procedure (cDNA synthesis, PCR amplifying, cloning, and sequencing) was repeated using primers which terminated before the codons in question. This showed that Ser and Pro were encoded at positions 7 and 8, respectively. The initial PCR product was rePCRred, using a sense primer which encoded Ser and Pro rather than Tyr and Gln at positions 7 and 8.

For each V gene, 2 PCR primers were synthesized. The amplification of genes by PCR, cloning into Puc18, as well as the double stranded DNA sequencing were all done as described above. The PCRred V genes were then cloned into the expression vectors PNY 1.16 and PGK11 which have human constant regions (Fig. 9 and 11) before transfection into the mouse myeloma cell line, Ag8.653.

The VL and VH genes were inserted into pGk.11 and pNy1.16, respectively, by amplifying the genes by PCR adding restriction site and intron sequences at the 5' and 3' ends.

The sense primers for both the H and L chains contained within their sequences the following in the 5' - 3' direction:

1. N6,
2. restriction site for cloning (EcoRI and SacI for the H chain; EcoRI and HindIII for the L chain; EcoRI is for cloning into pUC18, SacI and HindIII are for cloning into the expression vectors),
3. branch point lariat signal,
4. polypyrimidine tract,
5. splice acceptor site,
6. leader peptide 2,
7. the beginning of the FR1 of the V gene.

The reverse complement of the antisense primer contained:

1. 3' end of VDJ gene for the H chain and of the VJ gene for the L chain,
2. splice donor signal,
3. restriction site for cloning (Sall and XhoI for both H and L chains; Sall is for cloning into pUC 18, XhoI is for cloning into the expression vectors),
4. N6.

The sense primer for the heavy chain was:

5'**a**<sub>6</sub>GAATTTCGAGCTCTTTTCTGATAACGTTGTCCTTCTGTTTCTTGCAGGT  
GTCCAGTGTCAAGTCCAACCTTCAGCAGCCTGGG3'

The anti-sense primer for the heavy chain was:

5'**A**<sub>6</sub>GTTCGACCTCGAGTGTGAGGACTCACCTGAGGAGACGGTGACTGAGGT  
GCCT3'

The sense primer for the light chain was:

5'**a**<sub>6</sub>GAATTCAAGCTTTCCTGACTACATGAGTGCATTTCTGTTTTATTTC  
ATTTCAGATAACACCGGAGACATTGTGCTAACACAATCTCCA3'.

The anti-sens primer for the light chain was:

5' A<sub>6</sub>GTCGACCTCGAGATCACTTACGTTTGATTTCAGCTTGGTGCCTCCAC  
3'

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The amplification of genes by PCR, cloning into pUC18 and the double stranded DNA sequencing were done as described above. The PCR'd V genes were then cloned into the expression vectors. For the heavy chain, the V gene was directionally cloned into the Sac I and Xho sites of pNy1.16. For the light chain, the V gene was directionally cloned into the HindIII and Xho I sites of pGk.11.

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Humanized VH and VL genes were constructed by oligos and is described in detail herein below. The insertion of humanized V<sub>H</sub> and V<sub>L</sub> genes into pNy1.16 and pGk.11, respectively were done by the procedures described for the chimeric Ab. The sequences needed for cloning and expression were built in (or included in) in oligo #1 and #10.

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The presence of the chimeric 60.3 Abs were detected by ELISA. In this assay, 96 well plates were coated with goat anti-human IgG. The chimeric 60.3 in the sample which bound to the plates were detected by using horse radish peroxidase (HRPO) conjugated human anti-kappa IgG. Purified chimeric L6 (an unrelated anti-tumor antibody) was used as a standard. The culture supernatants from Ab producing clones were selected for binding in FACS binding assays.

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#### FACS Binding Assays

HL60 is a human myelomonocytic cell line which expresses CD 18 on the cell surface.

HL60 target cells, grown in culture media (DMEM, 1% fetal calf serum and 2% L-Glutamine), were made 10<sup>7</sup>/ml in the culture containing 0.1% NaAzide at 4°C. 10<sup>6</sup> cells were used per binding assay. Bovine IgG (Sigma, 20 ug/ml final concentration) was added for 10 min. at 4°C to mask the Fc receptors before performing the assays.

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Binding assays were done as direct binding, pre-incubation or competition assays.

#### 1) Standard binding assays

Various amounts of murine, chimeric or humanized 60.3 were incubated for 45 minutes with HL60 cells, washed and then incubated with FITC-labeled goat anti-mouse (for murine 60.3) antibody (Tago, Inc.) at 1:50 dilution or FITC-labeled goat anti-human antibody (for chimeric and humanized 60.3.) The fluorescence intensity was then monitored.

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#### 2) Pre-incubation assays

Pre-incubation experiments were done with murine 60.3. For pre-incubation with either chimeric 60.3, or humanized 60.3, 1000 ng/ml of either c60.3 or h60.3 was added to the HL60 cells for 45 minutes to 1 hr at 4°C. Cells were then washed twice with 1 ml of ice cold NaAzide-containing culture media and centrifuged in a Beckman table top centrifuge (Model TJ-6) at 1000 rpm for 5 min. After decanting washes, cells were resuspended in various concentrations of FITC-conjugated murine 60.3 Abs and incubated on ice for 45 min. to 1 hr. with intermittent mixing at 10min intervals. Cell pellets were fixed in 300 ul of 1 % paraformaldehyde at 4°C before analysis of FACS machines.

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#### 3) Co-incubation assays

Co-incubation assays were carried out by incubating various concentrations of chimeric 60.3 or humanized 60.3 with a saturating concentration (1 ug) of FITC-conjugated murine 60.3 Abs. Both types of Abs were added to the HL60 cells simultaneously; thus eliminating one incubation step. Incubation, washing and centrifugation were all done as in the pre-incubation assays. The cells were also suspended in 1 % ice cold paraformaldehyde before analysis by FACS.

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The mean channel values obtained from FACS analyses were translated into linear fluorescence equivalence (LFE) values. The LFE values were further used to calculate the fluorescence intensity levels (FIL) according to the following equation:

$$FIL = LFE \text{ of sample} / LFE \text{ of negative control (no second antibody)}$$

Electroporation of cells was carried out on a BioRad electroporator, set on capacitance of about 960 fu and .25 volts. A count of viable cells was taken before starting. Cells were at least 90% viable for use. Cells were also in the 4-6 x 10<sup>5</sup> /ml range; if they are overgrown, they will not show high transfection efficiency. 1 x 10<sup>7</sup> cells/electroporation group were removed and one group was used for a control electroporation and one for an "unzapped" control. Cells were centrifuged at 1000 rpm for 10 minutes. Supernatant was removed by vacuum with a sterile, unplugged pasteur pipet and the pellet was resuspended in as large a volume of PBS as the tube will allow and washed again.

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The pellet was resuspended in 0.8 ml PBS per 1 x 10<sup>7</sup> cells and 0.8 ml aliquot were added into labeled cuvettes. 10 ug each of DNA was added to cuvette. Incubate cuvette on ice for 10 minutes after mixing. The cuvettes were electroporated noting time factor reading and put on ice for 10 minutes, and then transferred



into 19 mls of IMDM/10% FBS, using 1 ml of medium to wash them out of the cuvettes. Cells were at 37° C for 48 hours, and then plated at  $10^4$ ,  $3 \times 10^3$ , and  $1 \times 10^3$  cells per ml in IMDM with 10% FBS and fed for 2-3 weeks before screening.

Enzyme-linked immunosorption assays (ELISA) were carried out as is known in the art. In an illustrative embodiment of ELISA in the present invention was performed as follows. Plates were created by diluting goat anti-human IgG 1:10,000 with 0.05M carbonate buffer, pH 9.6 and transferring 100  $\mu$ l to each well of a 96 well microtiter plate. The plate was then incubated at 4°C for 12 to 16 hours. The plates were then rinsed 1 to 3x and specimen diluent was added. The plates were then incubated at room temperature for 1 hr and rinsed 3x. On separate plates, 30  $\mu$ l of cultural supernatant was diluted to 300  $\mu$ l with the specimen diluent and 50  $\mu$ l was transferred to the previously coated plates. 50  $\mu$ l of specimen diluent was added and maintained at room temperature for 1 to 2 hours and rinsed 3x. HRP-conjugated human anti kappa was diluted with conjugate diluent 1:5,000 and 100  $\mu$ l was added per well to plates and incubated at 37°C for 30 minutes and washed 3x. A chromagen (1:300) with buffer substrate, pH 5.5 (room temperature) and 100  $\mu$ l was added to the plates. The plates were incubated at room temperature for 15 minutes, 100  $\mu$ l of 3M  $H_2SO_4$  was added and the plates were read at a wavelength of 450 nm and 630 nm.

Chimeric and humanized Mab 60.3 were analyzed using size exclusion HPLC (sechPLC), sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and isoelectric focusing (IEF). Test samples were compared to a murine 60.3.

#### sechPLC

A TSK3000SW Spherogel 7.5 x 600 mm column manufactured by Toso Haas was used. The mobile phase was 0.05 M phosphate buffered saline and samples were eluted at 0.5 ml/minute for 60 minutes. The chromatograms show that the test lots have multiple contaminating peaks and a major peak eluting at about 27.5 minutes. The major peak observed with the reference murine 60.3 Mac chromatogram, eluted at about 26.7 minutes.

#### SDS-PAGE

SDS-PAGE was performed using a 4-20% gradient gel and bands were detected by Coomassie blue staining. All lots were compared to the murine reference lot. Samples were run both reduced with 2-mercaptoethanol and non-reduced. Non-reduced gels showed that the major band ran consistent with the reference standard. The reduced gel showed data consistent with heavy and light chain separation.

#### IEF

Isoelectric focusing was performed using precast gels with a pH range of 3-10. Both 2  $\mu$ g and 5  $\mu$ g of sample were applied and run at 100 volts for 60 minutes, 200 volts for 60 minutes, and 500 volts for 30 minutes. Gels were stained with Coomassie Blue R-250. The chimeric 60.3 sample had no visible bands. This suggests that the material did not migrate into the gel. The humanized 60.3 sample had visible bands at the top of the gel in a region difficult to determine pI's. This data suggest that the chimeric and humanized lots of antibody have pI's greater than 8.0 as determined by the reference markers. This is in contrast to the murine mAb 60.3 which has a pI range of 6.8 - 7.5.

Having described this invention and embodiments thereof, the present invention is further illustrated by the following Examples which are not intended to limit the scope of the invention.

#### EXAMPLE 1: Comparative Model Building

The structural templates for comparative model building of murine 60.3 were determined. The Brookhaven Protein Data Bank was searched for the sequences with known structure which are most homologous to murine 60.3 heavy and light chains. The antiphosphocholine murine myeloma antibody, McPC603 (Satow et al. (1986) J. Mol. Biol. 190 : 593-604) was found to be the most homologous to the light chain, with 68% sequence identity/homology. The anti-p-azophenylarsonate murine monoclonal IgG2, R19.9 (Lascombe et al (1989) Proc. Natl. Acad. Sci. USA 86 : 607-611), was found to be the most homologous to the heavy chain, with 59% sequence identity/homology. MCPC603 VL and R19.9 VH were combined in order to obtain the structural template for model building of murine and humanized 60.3.

The CDR loops and known structural determinants of murine 60.3 were determined. Three CDR loops in 60.3 can be directly assigned to known canonical types (Chothia et al (1989) Nature 342 : 377-383). These

are L2 (type 1), L3 (type 1) and H1 (type 1). The remaining CDR loops do not belong to known canonical structural types. The boundaries of these loops can be determined by aligning the 60.3 sequence with that of the structural model. These assignments are shown along with those of L2, L3 and H1 in Figs. 1 and 2. The framework residues which are crucial for the conformations of the CDR loops are also shown in Figs. 1 and 2 (for example, \* L1 indicates structural determinants for the L1 loop) and in Sequence I.D. Numbers 2, 4, 6, and 8.

A model of murine 60.3 was then built. Backbone loop templates for L2 and L3 were taken from McPC603, and H1 from R19.9. L1 was initially modeled by a two residue deletion of the L1 loop of McPC603. H2 was found to be similar to the corresponding loop region in R19.9, which may represent a not yet classified canonical motif.

No loop closely related to H3 was found in the Brookhaven database. As a initial approximation for H3, an antibody non-CDR loop of the same size as 60.3 H3 (defined here as 96 to 102 in the Kabat numbering scheme or as H99 - H109 in the continuous sequence) was used. The backbone template for this loop was a 13 residue segment of the antibody NEW (Poljak RE. et al. (1974) Proc. Natl. Acad. Sci. USA 71 : 3340), beginning at residue L8. The loop was selected because it has the same length and showed a reasonable fit into the adjacent framework of H3. Energy minimization/conformation refinement of murine 60.3 resulted in: 1) residual rms derivatives of the energy function : 0.63 kcal/mol Å 2) Backbone rms deviations from the initial crystal coordinates:  $V_L$ : 0.86 Å ;  $V_H$ : 1.17 Å.

The most homologous human variable region sequences were found. The human sequence most homologous to 60.3  $V_L$  is PIR Accession # A01900 (sequence identity/homology 86%). This is the Vg germline sequence described by Pech and Zachau (Pech, M. and Zachau, H.G. (1984) Nucleic Acids Res. 12 : 9229-9236). Vg belongs to human Vk subgroup IIIa.

The human sequence most homologous to 60.3  $V_H$  is PIR Accession # A32483 (homology 59%). This is the heavy chain from human monoclonal Ab MO30 (anti-HIV gp 120) (Larrick et al. (1989) BBRC 160 1250-1256). There are two germline sequences highly homologous (1 aa mismatch through FR3) to MO30 : 21-2 and 3-1 (Berman et al (1988) EMBO J. 7: 727-738). These sequences belong to human VH subgroup 1.

The structural template for murine 60.3 was confirmed to be suitable for the human template. The human template for the heavy chain (MO30) is 56% homologous to R19.9. The human template for the light chain (Vg) is 62% homologous to McPC603. These numbers are similar to the homology between murine 60.3 and the same structural templates. The CDR loops and the structural determinants for the human template are shown in Figs. 1 and 2. The CDR loops and known structural determinants were then grafted onto the human template (Jones et al. (1986) Nature 321 : 522-535). The results are shown in Figures 1 and 2 in the column marked Phase I h60.3. In these figures, the h60.3 sequences and all identical sequences from the other columns are shaded.

A Phase 1 model of humanized 60.3 was then built using the same model building protocol as for murine 60.3. The backbone CDR loop templates were the same as for m60.3. Energy minimization/conformational refinement of murine 60.3 and Phase I h60.3 resulted in:

residual rms derivatives of the energy function:

m60.3 : 0.63 kcal/molÅ

Phase I h60.3 : 0.58 kcal/molÅ

Backbone rms deviations from the initial crystal coordinates:

m60.3 model  $V_L$ : 0.86 Å;  $V_H$ : 1.17 Å

Phase I h60.3  $V_L$ : 0.96 Å;  $V_H$ : 1.15 Å

In Phase II, the murine and Phase I h60.3 models were compared and refined. A comparison of the models of murine and humanized 60.3 gave an rms deviation of 0.66 Å and 0.78 Å for  $V_L$  and  $V_H$ , respectively. As a result of the comparison, it was postulated that certain residues which were still "human" were important for the conformation of the CDR loops. These were therefore changed to the murine residues, and are shown in bold in the column marked Phase II/III h60.3 in Figures 1 and 2. Sequence comparisons of m60.3 and Phase II 60.3 models (including the CDR loops) gave the following homologies:

Phase II 60.3  $V_L$  :

vs. murine 60.3  $V_L$  82%

vs. human template  $V_L$  82%

Phase II h60.3  $V_H$

vs. murine 60.3  $V_H$  78%

vs. human template  $V_H$  81%

Phase II h60.3  $F_V$

vs. murine 60.3  $F_V$  80%

vs. human template  $F_V$  81%

In Phase III further improvements of the structural model of h60.3 were made. After further refinement of the murine and humanized models, the following parameters were obtained:

Final rms derivatives of the energy function:

murine 60.3 model: 0.55 kcal/mol Å

5 Phase III h60.3: 0.78 kcal/mol Å

Backbone rms deviations from the initial crystal coordinates:

murine  $V_L$ : 0.82 Å,  $V_H$ : 1.09 Å  $F_V$ : 0.98 Å

Phase III h60.3  $V_L$ : 0.88 Å,  $V_H$ : 1.07 Å  $F_V$ : 1.00 Å

10 Finally, in Phase IV, the binding site features of the Phase III h60.3 model were analyzed. When comparing murine vs. humanized models at previous stages, the emphasis was more on the comparisons of the CDR - framework interactions in the murine antibody and the humanized contacts. The Phase IV model, where the L1 and H3 loops were remodeled using conformational search, allows a more detailed analysis of the CDR surface than the previous models.

15 Analysis of the model suggests that 60.3 is a distinct groove-type antibody and that certain CDR loops (L2 and H1) may not be involved in antigen binding. In the Phase IV model, the amino acids in these loops have been changed to the sequences from the human template.

## EXAMPLE 2

### 20 CONSTRUCTION OF THE HUMANIZED 60.3 $V_H$ AND $V_L$ GENES

Construction of the humanized 60.3 V genes was done according to the modeling methodology described in Example 1 by piecing 5 pairs of complementary oligos together (see Figures 3 and 4 and Sequence I.D. Numbers 11 to 30) each oligo was about 90 to 100 nt in length, together they span the entire V regions, forming the  $V_H$  and  $V_L$ . The step by step protocol used was as follows.

- 25 1. Five microfuge tubes were labeled and the following oligos (10 ng each, 10 ul) were added:
  - tube 1: oligo #2
  - tube 2: oligo #3 and #4
  - tube 3 : oligo #5 and #6
  - 30 tube 4 : oligo #7 and #8
  - tube 5 : oligo #9
2. Tubes 2, 3 and 4 were heated to 100°C for 3 min. and then cooled slowly to room temp.
3. To all 5 tubes, 10x kinase buffer (40 mM Tris, Cl pH7.5, 10 mM  $MgCl_2$ , 10mMDTT, 1ug DNA, 0.5 mM ATP, 50 ug/ml bovine serum albumin and 1 "weiss" unit T4 DNA ligase), 100 uM ATP (1 ul) and nucleotide kinase
- 35 were added to phosphorylate the 5' ends of the oligos. One Weiss unit is equivalent to 60 cohesive-end units. Reactions proceeded at 37° for 1 hr.
4. The tubes were extracted with phenol/ $CHCl_3$  and precipitated with ethanol.
5. Oligos #1 and #10 (10ng, 10 ul) were added to tubes 1 and 5, respectively. Tubes 1 and 5 were mixed and step 2 was repeated for tubes 1 and 5.
- 40 6. The contents from all 5 tubes were pooled into a single tube.
7. Ligate at 12° C, for 12 to 16 hours in a vol. of 25 ul.
8. Analyze 2 ul on 1.0 % agarose.
9. After successful ligation, restriction digest an aliquot with EcoRI and Sall for 45 min. at 37°C.
10. Apply onto 3.0% low melt agarose gel and cut out the correct sized band (approximately 0.4kb).
- 45 11. Ligate into Puc 18 (pre-digested with EcoRI and Sall).
12. Transform DH5α cells.
13. Select the potential positives based on Xgal/IPTG indicators.
- 14 Miniprep cultures were prepared and maintained for 12 to 16 hours.
15. Plasmid DNA was isolated from these cultures and the insert sizes were checked by cutting with EcoRI and Sall.
- 50 16. The plasmid DNA was sequenced for verification. Several of the clones had mutations such as single base deletions.
17. The synthetic H and L variable genes were cloned into appropriate expression vectors (pNG1.16 and pGk. 11, respectively.)
- 55 18. The potential positives were isolated after selection on ampicillin.
19. Steps 14 to 16 were repeated.
20. Transfection into mouse Ag8.653 myeloma cells was carried out, followed by selection with G418 (Raff, et al., (1991) J. Infect. Dis. 163 : 346-354).

21. Ig positives were then screened with ELISA (gamma, kappa capture). The DNA sequences of the murine 60.3 heavy and light chains are shown in Sequence I.D. numbers 5 and 7, respectively; and the DNA sequences for the humanized 60.3 H and L chains are shown in Sequence I.D. numbers 1 and 3, respectively.

### EXAMPLE 3

#### BINDING ASSAYS OF 60.3

The binding activity of the humanized 60.3 antibody was measured by preincubation, competition and chemiluminescence assays.

HL60 is a human myelomonocytic cell line which expresses CD 18 on the cell surface.

HL60 target cells, grown in culture media (DMEM, 1% fetal calf serum and 2% L-Glutamine), were made  $10^7$ /ml in the culture media containing 0.1% NaAzide at 4°C.  $10^6$  cells were used per binding assay. Bovine IgG (Sigma, 20 ug/ml final concentration) was added for 10 minutes at 4°C to mask the Fc receptors before performing the assays. Three types of binding assays were performed.

##### A) Standard curves

As illustrated in Figure 5, various amounts of murine, chimeric, and humanized 60.3 were incubated for 45 minutes with HL60 cells. The cells were washed and then incubated for 45 minutes with either FITC conjugated goat anti mouse IgG (for m60.3) or FITC conjugated goat anti human IgG (for c60.3 and h60.3). Excess antibody was washed off and the cells were fixed with 1% paraformaldehyde and assayed by FACS. For each curve, the value obtained at 1500 ng/ml is taken as 100%. Data at other concentrations are plotted as % of this value. As can be seen in Figure 5, all three antibodies titrate over approximately the same range., indicating similar affinities (known to be about  $10^9$  for m60.3).

##### B) Preincubation experiments

HL60 cells were preincubated for 45 minutes with 1 µg/ml of either c60.3 or h60.3. The indicated amount of FITC conjugated m60.3 (or no antibody) was then added and the cells were incubated for another 45 minutes. After washing, the cells were fixed with paraformaldehyde and assayed by FACS. As can be seen in Figure 6, both the chimeric and humanized antibodies were able to completely block the binding of FITC - m60.3 to HL60 cells (note FIL = 1 is equivalent to no binding).

##### C) Competition experiments

As illustrated in Figure 7, 1 µg of FITC conjugated m60.3 and the indicated amount of either c60.3 or h60.3 were coinubated with HL60 cells for 45 minutes. The cells were washed, fixed with paraformaldehyde and assayed by FACS. As can be seen in Figure 7, the chimeric and humanized antibody competed equivalently in this assay. The dashed line shows binding of 1 µg FITC - m60.3 in absence of competitor.

#### 3) Chemiluminescence Assay

An assessment of activity of 60.3 by inhibition of Zymosan-induced, luminol-enhanced chemiluminescence of PMN was carried out. The material and compositions and procedures used were as follows.

##### Preparation of Components:

##### 1. GGVB

###### 1.1. Materials

a) 5x Veronal: Dissolve 41.2g NaCl and 5.095g 5,5-diethylbarbiturate (Paragon B-2 buffer) in 700 ml deop-moz d (diH2O). Adjust pH to  $7.35 \pm 0.05$  with 1N HCl. Bring volume to 1 liter with diH2O. Filter sterilize and store at 4°C. Stability but is at least 2 months.

b) Stock materials: mix equal volumes of 2M MgCl<sub>2</sub> (40.66g/100ml) and 0.3M CaCl<sub>2</sub> (4.4g/100ml). Filter sterilize and store at 4°C. Stability is at least 6 months.

- c) gelatin
- d) dextrose

## 1.2. Procedure

5

For 300 ml GGVB:

Add 0.3g gelatin and 0.3g dextrose to 240 ml diH<sub>2</sub>O. Heat with mixing until just dissolved. Let cool to below 37C.

Add 60 ml 5x Veronal and 0.3 ml stock metals. Filter sterilize and store at 4C.

10

This is made up fresh for each day's assays.

## 2.2. Zymosan

### 2.1. Materials:

15

- a) Zymosan A, SigmaZ-4250
- b) diH<sub>2</sub>O
- c) 1 x PBS

20

### 2.2. Procedure:

Suspend zymosan to 25 mg/ml (1.5g/60ml) in diH<sub>2</sub>O. Heat in glass in a water bath at 100C for 60 min.

Transfer to 50 ml polypropylene centrifuge tubes. Centrifuge (8500 rpm in the TJ-6, 10 min, RT) and wash twice with 1 x PBS. Resuspend to 50 mg/ml (1.5g/30ml) in 1 x PBS. Store at 4C. Stability is at least 2 months at 4C.

25

## 3. Complement (adsorbed human serum)

### 3.1. Material:

30

a) Freshly drawn human blood, without anticoagulants. Type O may be best, or use the same donor that supplies PMN. Transfer blood immediately after drawing into sterile 50ml glass centrifuge tubes (available from blood lab).

35

b) 0.1 M EDTA, pH 7.35. Dissolve 3.72g Na<sub>2</sub>H<sub>2</sub>EDTA in 80 ml di H<sub>2</sub>O, with mixing. Adjust pH to 7.35 ± 0.05 with freshly prepared 2N NaOH. Bring to 100 ml volume with diH<sub>2</sub>O.

c) Zymosan prep (see 2.1)

### 3.2. Procedure:

40

Allow blood to clot at room temperature for 1 hour. Rim the tube with a glass pipet to contract the clot. Centrifuge (8500 RPM in TJ-6, 20 min., RT) and carefully remove serum to a sterile polypropylene tube. Repeat centrifugation if necessary.

Add 0.1 M EDTA to serum to 10% mg/ml zymosan needed to adsorb serum at 0.2 mg zymosan/ml of serum. Add this volume of zymosan to each of four centrifuge tubes. Centrifuge zymosan tubes (8500 rpm in the TJ-6, 10 min., RT) and remove supernatant. Keep the tubes on ice.

45

Resuspend the zymosan pellet of one tube with a small amount of serum. Add the remainder of the serum and mix by inversion.

Incubate the serum/zymosan mixture on a rocker or rotator, 30 min, 4C.

Centrifuge as above. Repeat the adsorption with the other three tubes.

50

After the final centrifugation, filter the serum through a 0.45µm syringe filter Aliquot into microfuge tubes or equivalent and store at -70C. The adsorbed serum has an unknown stability but it probably good for at least six months.

## 4 Human PMN

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### 4.1. Materials

- a) Freshly drawn human parinized blood.

- b) Ficoll-Hypaque Mono-Poly Resolving Medium (MPRM), Flow Labs no. 16-980- 49
- c) 1 x PBS
- d) 3% HoAc in diH<sub>2</sub>O
- e) Serum-free, phenol red-free RPMI with 0.5% gelatin (RPMI-g I): Add gelatin (1.25g/250 ml) to serum-free, phenol red-free RPMI and heat with stirring until gelatin is just dissolved. Filter sterilize (0.45 µm filter) and store in 50ml aliquots at 4°C. Stability is least 2 weeks.

#### 4.2. Procedure

- 10 Aliquot 4 ml MPRM into 15 ml polypropylene centrifuge tubes.  
Overlay gently with 5 ml fresh blood.  
Centrifuge (30 min, 2000 rpm in TJ-6, RT). If RBC are not completely pelleted, centrifuge an additional 10 minutes. If no RBC sedimentation has occurred, additional centrifugation will not work and another donor must be found.
- 15 Aspirate top (plasma) layer and first layer of cells (monocytes).  
With a Pasteur pipet, carefully remove the second cell layer (PMN) to a 50ml centrifuge tube. Repeat with all tubes.  
Add RT at room temperature, 1 x PBS to the PMN tube to a final volume of 50 ml and mix gently.  
Remove 50µl and dilute appropriately in 3% HoAc (usually 1:20 is appropriate for a prep of 30ml whole blood) and count PMN in a hemocytometer. Calculate total PMN present in the 50ml tube.
- 20 Centrifuge PMN (10 min, 2000 rpm in TJ-6, RT) and resuspend to desired density (normally  $2 \times 10^6$ /ml) in RPMI-gel. Store at RT, swirling gently to resuspend occasionally (once or twice an hour). Viability should be > 90% after 6 hours; if used after 6-8 hours, check viability by trypan blue exclusion prior to use.

#### 25 5 Luminol

##### 5.1. Materials

- 30 a) Luminol (5 amino-2,3 dihydro - 1,4 - phthalazinedione), Sigma no. A-8511.  
FW=172.2
- b) DMSO, chromatography grade

##### 5.2. Procedure

- 35 Dissolve luminol to  $10^{-2}$ M in DMSO (17.7 mg/10ml). Store at 4°C in the dark (wrap tube in foil). Stability is more than 1 month; make fresh 3-4 weeks.

#### 6. Antibody

##### 40 6.1. Materials and Procedure

Dilute antibody in GGVB to appropriate concentrations.

##### Assay Design

- 45 Test volumes per tube are normally 1 ml, composed of the following.
- 100 µl antibody (sensitivity to concentration is unknown, previous work has been with 5 µg/ml preps)
- 100 µl PMN (normal concentration at  $2 \times 10^6$ /ml)
- mix by swirling gently and incubate 30 min, at room temperature (RT) (cover all tubes with one sheet of Parafilm)
- 50 - 100 µl Zymosan (high concentrations, around 50mg/ml, seem to work best)
- just before loading luminometer, add:
- 600 µl luminol (diluted in GGVB, normal concentrations are  $10^{-4}$  or  $5 \times 10^{-5}$ M)
- 100 µl complement (low concentrations, on the order of 1-2%, seem to work better)

55

##### Staging of Assay

Set up CL tubes (Clinicon 2174-089, available through LKB). Keep in the dark (in a drawer) to prevent spon-

taneous luminescence from absorbed fluorescent light.

Thaw complement at RT and hold on ice, just prior to assay complement may be unstable (may lose 50% of activity in 6-8 hours) at 4°C.

Add antibody, PMN, and zymosan as described above to CL tubes.

5 Take tubes to luminometer. Program assay parameter into controlling computer.

Prepare final dilution of Luminol (and keep wrapped in foil) just before adding to CL tubes.

Prepare final dilution of complement in GGVB.

Add luminol and complement to CL tubes as described above and load into luminometer. Start the program immediately. Peak luminescence is reached 4-5 minutes after adding complement.

10 Throughout assay setup and during the first rotation of the CL tubes in the luminometer, apply anti-static charge with the anti-static gun to prevent CL tubes hanging up in the luminometer.

## Results

15 The above procedure was utilized to measure the ability of murine, chimeric, and humanized 60.3 to inhibit CR3(CD11b/CD18) mediated uptake of opsonized zymosan by neutrophils. In the absence of 60.3, phagocytic uptake of opsonized zymosan results in an increase in hexose monophosphate shunt activity that is measured as light output by luminol-enhanced chemiluminescence.

20 
$$\% \text{ inhibition of chemiluminescence signal} = 100 \times (1 - \text{signal of sample} / \text{signal of negative control antibody})$$

The results shown in Figure 8 illustrate that all of the 60.3 antibodies were reactive in this assay, while an irrelevant antibody (murine L6 anti-tumor antibody) showed no reactivity.

## EXAMPLE 4

25

### CONSTRUCTION OF pGK.11

In order to express either chimeric or humanized light chains, cassette vectors were constructed capable of expressing variable region genes, synthesized using PCR (polymerase chain reaction). In addition to sequences found on pSV2-gpt, these vectors contain an Ig promoter and leader, the 5' portion of an intron, a MCS for insertion of the variable region gene, the mouse heavy chain enhancer (MHE), the 3' portion of an intron and the human Ck gene. The variable region was PCR'd so that in addition to the coding region it contained flanking intron sequences.

35 9): The cassette for the expression of the light chain was constructed as follows (and is illustrated in Figure 9):

1. The 121 bp Hind III to Bgl II fragment of pSV2-gpt (Mulligan and Berg (1980) Science 209: 1422) was deleted from the 5' end of the Ecogpt by restriction with Hind III and Bgl II, filling in with Klenow polymerase, and religation. The product, pG.2, was detected by the absence of Hind III and Bgl II sites and linearization with EcoR I.

40 2. The pBR322 part of pG.2 (EcoR I-Pvu II fragment) was replaced with the analogous portion of pUC 18 (Pvu II - Pvu II fragment) to form pG.3. This product was screened for by digestion with Pst I, which linearizes pG.3 giving a 5.05 kb fragment.

3. pG.3 was made into pG.5 by the replacement of the 750 bp EcoR I to BamH I fragment with the 64 bp EcoR I to BamH I multiple cloning site (MCS) from pC20R (Marsh et al. 1984). pG.5 was screened for the presence of an Xho I site (part of the MCS).

45 4. A Not I site was inserted in the Nde I site of pG.5 to form pG.12. Oligonucleotide linkers (Sequence I.D. numbers 33 and 34) were used for this purpose. pG.12 was screened for the presence of a Not I site.

5. A 2.75 kb EcoR I fragment containing the human Ck gene was inserted into the EcoR I site of the MCS of pG.12 to form pGk.3. When the Ck fragment was in the correct orientation, Sac I digestion produced 0.126, 0.509 and 6.7 kb fragments (vs. 0.509, 2.1 and 4.7 kb fragments in the wrong orientation).

6. A 140 bp portion of the SV140 enhancer was removed from pG.3 by restriction with Sph I, destroying the overhang with the exonuclease activity in Klenow polymerase, followed by digestion with Pvu II and blunt end ligation. The product, pG.9, was screened for the loss of Pvu II, Nsi I and Sph I sites.

7. A Not I site was inserted in the Nde I site of pG.9 to form pG.10. Oligonucleotide linkers, described in #4 above, were used for this purpose. pG.10 was screened for the presence of a Not I site.

55 8. The 195 bp Nde I to BamH I fragment from pG.12 was inserted into the Nde I to BamH I site of pG.10 to form pG.11. This served to place an 879bp fragment from pG.10 with a 195 base pair fragment containing a MCS. pG.11 was screened for the presence of Xho I site in the MCS region.

9. The 3kb Nar I to Cla I fragment from pGk.3 was directionally subcloned into the same sites of pG.11 to form pGk.4.

10. A 1kb fragment containing the mouse heavy chain enhancer was transferred from pICMHEXX to pGk.4 as a Cla I to Hind III fragment, thus forming pGk.5. EcoR I digestion of pGk.5. produced 0.3, 2.75 and 5.1 kb fragments. pICMHEXX was made by the insertion of the 1kb Xba I fragment (filled in with Klenow polymerase) from RBL 216 (Lang et al (1982) Nucl. Acid Res. 10: 611-620) into the filled in Bgl II site of pIC19 R (Marsh et al (1984) Gene 32: 481-486).

11. A 579 bp Sau3a I fragment containing the 489 promoter pGkA1.9 (Raff et al, 1991) was inserted into the BamH I site (in the MCS) of pGk.5 to form pGk.11. The resulting plasmids were screened for the correct orientation of the insert: BamH I plus Asp718 I digestion gave a 1.2 kb fragment in the correct orientation vs. a 2.2 kb fragment in the wrong orientation. Also, BamH I plus Hind III digestion should give a 0.58 kb fragment.

The sequence of specific regions is contained in the following segments. The sequence is given in clockwise orientation beginning at the EcoR I site at 0° and is illustrated in Figure 10 and Sequence I.D. 10.

The ampicillin resistance gene is bp 7383 to 8241

The ecogpt gene is bp 5651 to 6107

The mouse heavy chain enhancer is bp 2770 to 3788

The Sau3a I fragment containing the Alk promoter and leader is bp 3827 to 4393, with the leader peptide encoded by bp 3951 to 3999.

The EcoR I fragment containing the human Ck gene is bp 6 to 2756, with the Ck region itself encoded by bp 2113 to 2435.

## EXAMPLE 5

### Construction of pNy1.16

In order to express either chimeric or humanized heavy chains, cassette vectors were constructed capable of expressing variable region genes synthesized using PCR (polymerase chain reaction). In addition to sequences found on pSV2-neo, these vectors contain an Ig promoter and leader, the 5' portion of an intron, a MCS for insertion of the variable region gene, the mouse heavy chain enhancer (MHE), the 3' portion of an intron and the human C $\gamma$ 1 gene. The variable region gene is PCR'd so that in addition to the coding region it contains flanking intron sequences.

The cassette for the expression of the heavy chain was constructed as follows (as illustrated in Figure 11)

1. The Hind III site in pSV2-neo was removed by digestion with Hind III, fill in with Klenow polymerase and religation. The product, pN.1 was screened for the absence of the Hind III site.

2. The 750 bp EcoR I to BamH I fragment from pN.1 was replaced by a 64 bp EcoR I to BamH I multiple cloning site (MCS) from pIC20R to form pN.5

3. A PCR region was done on pICMHEXX in a manner that primers were chosen to delete the EcoR I site while generating a new MCS region. This resulted in a product consisting of: a) recognition sequences for EcoR V, Asp 718 I, Sac I and Xho I; b) a 695 bp of the mouse heavy chain enhancer from the 5'Xba I site to the EcoR I site; and c) recognition sequences for Hind III, Sal I and BamH I. This 723 bp PCR product was cloned into pIC20R to form pMHE.per.

4. The 723 EcoR V - BamH I fragment from pMHE.per was subcloned into the same sites in the MCS region of pN.5. This removed the previous MCS, while inserting the one associated with the MHE. The product, pN.8, was screened for the presence of 0.7 and 5.0 kb Xho I - Hind III fragments and for linearization to 5.7 kb with EcoR I.

5. Two PCR SOEing (Horton et al, (1990) Biotech. 8 : 528-535) reactions were used to create several mutations in the L6 heavy chain promoter. Outer primers had enzyme sites EcoR V and SacI for subcloning into pIC20R to form pMUTL6HCP. The sequence of this insert is shown as bp 7793-8495 in Fig.12 and in Sequence I.D. number 9.

6. The 703 bp EcoR V to SacI fragment from pMUTL6HCP was inserted into the same sites of pN.8 to form pN.9.

7. A 3.5 kb Xho I - BamH I fragment from pNy1A2.5, containing the PCR'd MHEXR (Sequence I.D. Number 31) plus the 2.8 kb Hindm - BamH I fragment encoding the human  $\gamma$ 1 gene (Sequence I.D. Number 32) was inserted into the same sites of pN.9 to form pNy1.16. The sequence of this insert is shown as bp 2-2799 in Fig. 12 and in Sequence I.D. Number 9.

The foregoing description and the Examples are intended as illustrative and are not to be taken as limiting. Still other variations within the spirit and scope of this invention are possible and will readily present themselves to those skilled in the art.



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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT:  
 (A) NAME: Bristol-Myers Squibb Company  
 (B) STREET: 345 Park Avenue  
 (C) CITY: New York  
 (D) STATE OR PROVINCE: New York  
 (E) COUNTRY: USA  
 (F) POSTAL CODE: 10154  
 (G) TELEPHONE: 206-728-4800  
 (H) FAX: 206-727-3601
- (ii) TITLE OF THE INVENTION: HUMAMIZED MONOCLONAL ANTIBODIES
- (iii) NUMBER OF SEQUENCES 34
- (iv) COMPUTER READABLE FORM:  
 (A) MEDIUM TYPE: Floppy disk  
 (B) COMPUTER: IBM PC compatible  
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (v) CURRENT APPLICATION DATA:  
 (A) APPLICATION NUMBER: EP93401328.5  
 (B) FILING DATE: 24-MAY-1993  
 (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:  
 (A) APPLICATION NUMBER: US07/888233  
 (B) FILING DATE: 26-MAY-1992  
 (C) CLASSIFICATION:

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 361 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGGTCCAAC TTGTCCAGTC CGGTGCCGAA GTTAAGAAGC CTGGCGCTTC TGTGAAGGTC 60

TCCTGCAAGG CTTCTGGCTA CACCTTCACC GACTACTGGA TGAAGTGGGT TCGACAGGCA 120

CCTGGACAGG GCCTAGAGTG GATGGGAAGG ATTGATCCTT CCGATAGTGA AACTCACTAC 180

AATCAGAACT TCCAGGGTAG GGTAACAATG ACCCGAGACA CATCCACCAG CACAGTCTAC 240

ATGGAACTCA GCAGCCTGCG ATCTGAGGAC ACCGCAGTCT ATTACTGTGC ACGAGGTGGA 300

CGGCTCGGTT CCTTTGCTAT GGACTIONTGG GGTCAAGGCA CCCTCGTCAC CGTCTCCTCA 360

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361

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30  
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 25 35 40 45  
 Gly Arg Ile Asp Pro Ser Asp Ser Glu Thr His Tyr Asn Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Gly Gly Arg Leu Gly Ser Phe Ala Met Asp Tyr Trp Gly Gln  
 100 105 110  
 Gly Thr Leu Val Thr Val Ser Ser  
 115 120

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 334 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAAATTGTGC TAACACAATC TCCAGCTACA TTGTCTTTGT CTCCAGGTGA GAGAGCCACT 60  
 CTATCCTGCA GAGCCAGTGA AAGTGTGTAT AGTTATGGCA ATAGTTTAT GCACTGGTAC 120

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CAGCAGAAAC CAGGACAGGC ACCAAGGCTC CTCATCTATC GTGCATCCAA CCTAGAAACT 180  
 GGTATCCCTG CCAGGTTTCAG TGGCAGTGGT TCTAGGACAG ACTTCACTCT CACCTATTCT 240  
 10 TCTCTAGAGC CTGAAGATTT TGCAGTGTAT TACTGTCAGC AAAGTAATGA GGATCCTCGG 300  
 ACGTTCGGTG GAGGCACCAA GGTGGAAGAG AAAC 334

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 15 (A) LENGTH: 111 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr  
 20 25 30  
 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro  
 35 40 45  
 30 Arg Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Thr Gly Ile Pro Ala  
 50 55 60  
 Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Tyr Ser  
 65 70 75 80  
 35 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95  
 Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Glu Lys  
 100 105 110

## (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 361 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 CAGGTCCAAC TTCAGCAGCC TGGGCCTGAC CTTCTGAAGC CTGGGGCTCC AGTGAAGCTG 60

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TCCTGCAAGG CTTCTGGCTA CACCTTCACC GACTACTGGA TGAAGTGGGT TAAGCAGAGG 120  
 CCTGGACGAG GCCTCGAGTG GATTGGAAGG ATTGATCCTT CCGATAGTGA AACTCACTAC 180  
 AATCAGAACT TCAAGGACAA GGCCACACTG ACTGTAGACA AATCCTCCAG CACAGCCTAC 240  
 ATCCAACCTCA GCAGCCTGAC ATCTGAGGAC TCTGCAGTCT ATTACTGTGC ACGAGGGGGA 300  
 CGGCTCGGGT CCTTTGCTAT GGACTACTGG GGTCAAGGCA CCTCAGTCAC CGTCTCCTCA 360  
 G 361

15

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gln Val Gln Leu Gln Gln Pro Gly Pro Asp Leu Leu Lys Pro Gly Ala  
 1 5 10 15  
 Pro Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30  
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile  
 35 40 45  
 Gly Arg Ile Asp Pro Ser Asp Ser Glu Thr His Tyr Asn Gln Lys Phe  
 50 55 60  
 Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80  
 Ile Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Gly Gly Arg Leu Gly Ser Phe Ala Met Asp Tyr Trp Gly Gln  
 100 105 110  
 Gly Thr Ser Val Thr Val Ser Ser  
 115 120

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(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 334 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10 GACATTGTGC TAACACAATC TCCAGCTTCT TTGGCTGTGT CTCTAGGGCA GAGGGCCACC 60  
 ATATCCTGCA GAGCCAGTGA AAGTGTGTAT AGTTATGGCA ATAGTTTAT GCACTGGTAC 120  
 CAGCAGAAAC CAGGACAGCC ACCCAAATC CTCATCTATC GTGCATCCAA CCTAGAATCT 180  
 15 GGGATCCCTG CCAGGTTTCAG TGGCAGTGGG TCTAGGACAG ACTTCACCT CACCATTAAT 240  
 CCTGTGGAGG CTGATGATGT TGCAACCTAT TACTGTCAGC AAAGTAATGA GGATCCTCGG 300  
 ACGTTCGGTG GAGGCACCAA GCTGGAAATC AAAC 334

## (2) INFORMATION FOR SEQ ID NO:8:

- 20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 111 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 25 (ii) MOLECULE TYPE: peptide  
 (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15  
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr  
 20 25 30  
 35 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45  
 Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala  
 50 55 60  
 40 Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn  
 65 70 75 80  
 Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95  
 45 Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

## (2) INFORMATION FOR SEQ ID NO:9:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 9201 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

	AAGCTTTCTG	GGGCAGGCCA	GGCCTGACCT	TGGCTTTGGG	GCAGGGAGGG	GGCTAAGGTG	60
	AGGCAGGTGG	CGCCAGCCAG	GTGCACACCC	AATGCCCCATG	AGCCCAGACA	CTGGACGCTG	120
15	AACCTCGCGG	ACAGTTAAGA	ACCCAGGGGC	CTCTGCGCCC	TGGGCCCAGC	TCTGTCCCAC	180
	ACCGCGGTCA	CATGGCACCA	CCTCTCTTGC	AGCCTCCACC	AAGGGCCCAT	CGGTCTTCCC	240
	CCTGGCACCC	TCTTCCAAGA	GCACCTCTGG	GGGCACAGCG	GCCCTGGGCT	GCCTGGTCAA	300
20	GGACTACTTC	CCCGAACC GG	TGACGGTGTC	GTGGAAGTCA	GGCGCCCTGA	CCAGCGGCGT	360
	GCACACCTTC	CCGGCTGTCC	TACAGTCCTC	AGGACTCTAC	TCCCTCAGCA	GCGTGGTGAC	420
	CGTGCCCTCC	AGCAGCTTGG	GCACCCAGAC	CTACATCTGC	AACGTGAATC	ACAAGCCCAG	480
	CAACACCAAG	GTGGACAAAC	GCGTTGGTGA	GAGGCCAGCA	CAGGGAGGGA	GGGTGTCTGC	540
25	TGGAAGCCAG	GCTCAGCGCT	CCTGCCTGGA	CGCATCCCGG	CTATGCAGCC	CCAGTCCAGG	600
	GCAGCAAGGC	AGGCCCCGTC	TGCCTCTTCA	CCCGGAGGCC	TCTGCCCGCC	CCACTCATGC	660
	TCAGGGAGAG	GGTCTTCTGG	CTTTTTCCCC	AGGCTCTGGG	CAGGCACAGG	CTAGGTGCCC	720
30	CTAACCACAG	CCCTGCACAC	AAAGGGGCAG	GTGCTGGGCT	CAGACCTGCC	AAGAGCCATA	780
	TCCGGGAGGA	CCCTGCCCCCT	GACCTAAGCC	CACCCCAAAG	GCCAAACTCT	CCACTCCCTC	840
	AGCTCGGACA	CCTTCTCTCC	TCCCAGATTC	CAGTAACTCC	CAATCTTCTC	TCTGCAGAGC	900
35	CCAAATCTTG	TGACAAAAC	CACACATGCC	CACCGTGCC	AGGTAAGCCA	GCCCAGGCCT	960
	CGCCCTCCAG	CTCAAGGCGG	GACAGGTGCC	CTAGAGTAGC	CTGCATCCAG	GGACAGGCC	1020
	CAGCCGGGTG	CTGACACGTC	CACCTCCATC	TCTTCTCAG	CACCTGAACT	CCTGGGGGGA	1080
	CCGTCACTCT	TCCTCTTCCC	CCCAAAACCC	AAGGACACCC	TCATGATCTC	CCGGACCCCT	1140
40	GAGGTCACAT	GCGTGGTGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAACTGG	1200
	TACGTGGACG	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC	1260
	AGCACGTACC	GTGTGGTCAG	CGTCTCACC	GTCTGACACC	AGGACTGGCT	GAATGGCAAG	1320
45	GAGTACAAAG	GCAAGGTCTC	CAACAAAGCC	CTCCAGCCC	CCATCGAGAA	AACCATCTCC	1380
	AAAGCCAAAG	GTGGGACCCG	TGGGGTGCGA	GGGCCACATG	GACAGAGGCC	GGCTCGGCCC	1440
	ACCCCTCTGCC	CTGAGAGTGA	CCGCTGTACC	AACCTCTGTC	CCTACAGGGC	AGCCCCGAGA	1500
50	ACCACAGGTG	TACACCCTGC	CCCATCTAG	AGAGGAGATG	ACCAAGAACC	AGGTCAGCCT	1560
	GACCTGCCTG	GTCAAAGGCT	TCTATCCCAG	CGACATCGCC	GTGGAGTGGG	AGAGCAATGG	1620

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	GCAGCCGGAG AACAACTACA AGACCACGCC TCCCGTGCTG GACTCCGACG GCTCCTTCTT	1680
	CCTCTACAGC AAGCTCACC GGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG	1740
10	CTCCGTGATG CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC	1800
	GGGTAAATGA GTGCGACGGC CGGCAAGCCC CCGTCCCCG GGCTCTCGCG GTCGCACGAG	1860
	GATGCTTGCC ACGTACCCCC TGTACATACT TCCCGGGCGC CCAGCATGGA AATAAAGCAC	1920
15	CCAGCGCTGC CCTGGGCCCC TGCAGAGACTG TGATGGTTCT TTCCACGGGT CAGGCCGAGT	1980
	CTGAGGCCTG AGTGGCATGA GGGAGGCAGA GCGGGTCCCA CTGTCCCCAC ACTGGCCCAG	2040
	GCTGTGCAGG TGTGCTGGG CCCCCTAGGG TGGGGCTCAG CCAGGGGCTG CCCTCGGCAG	2100
20	GGTGGGGGAT TTGCCAGCGT GGCCCTCCCT CCAGCAGCAC CTGCCCTGGG CTGGGCCACG	2160
	GGAAGCCCTA GGAGCCCCTG GGGACAGACA CACAGCCCCCT GCCTCTGTAG GAGACTGTCC	2220
	TGTCTGTGA GCGCCCCTGT CCTCCCGACC TCCATGCCCA CTCGGGGGCA TGCCTAGTCC	2280
	ATGTGCGTAG GGACAGGCC TCCCTCACCC ATCTACCCCC ACGGCACTAA CCCCTGGCTG	2340
25	CCCTGCCCCAG CCTCGCACCC GCATGGGGAC ACAACCGACT CCGGGGACAT GCACTCTCGG	2400
	GCCCTGTGGA GGGACTGGTG CAGATGCCCA CACACACACT CAGCCCAGAC CCGTTCAACA	2460
	AACCCCGCAC TGAGGTTGGC CGGCCACACG GCCACCACAC ACACACGTGC ACGCCTCACA	2520
30	CACGGAGCCT CACCCGGGCG AACTGCACAG CACCAGACC AGAGCAAGGT CCTCGCACAC	2580
	GTGAACATC CTCGGACACA GGCCCCACG AGCCCCACGC GGCACCTCAA GGCCCCAGAG	2640
	CCTCTCGGCA GCTTCTCCAC ATGCTGACCT GCTCAGACAA ACCCAGCCCT CCTCTCACA	2700
35	GGGTGCCCCT GCAGCCGCCA CACACACACA GGGGATCACA CACCACGTCA CGTCCCTGGC	2760
	CCTGGCCCAC TTCCCAGTGC CGCCCTTCCC TGCAGGACGG ATCCAGACAT GATAAGATAC	2820
	ATTGATGAGT TTGGACAAAC CACAAC TAGA ATGCAGTGAA AAAAAAGCTT TATTGTGAA	2880
	ATTGTGATG CTATGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC	2940
40	AACAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT TTTTAAAGC	3000
	AAGTAAACC TCTACAAATG TGGTATGGCT GATTATGATC TCTAGTCAAG GCACTATACA	3060
	TCAATATTC CTTATTAACC CCTTTACAAA TAAAAAGCT AAAGGTACAC AATTTTGTAG	3120
45	CATAGTTATT AATAGCAGAC ACTCTATGCC TGTGTGGAGT AAGAAAAAC AGTATGTTAT	3180
	GATTATAACT GTTATGCCTA CTTATAAAGG TTACAGAATA TTTTCCATA ATTTCTTGT	3240
	ATAGCAGTGC AGCTTTTCC TTTGTGGTGT AAATAGCAAA GCAAGCAAGA GTTCTATTAC	3300
50	TAAACACAGC ATGACTCAAA AAACCTAGCA ATTCTGAAGG AAAGTCCTTG GGGTCTTCTA	3360
	CCTTCTCTT CTTTTTGGG GAGTAGAAT GTTGAGAGTC AGCAGTAGCC TCATCATCAC	3420

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	TAGATGGCAT	TTCTTCTGAG	CAAAACAGGT	TTTCCTCATT	AAAGGCATT	CACCACTGCT	3480
	CCCATTTCATC	AGTTCCATAG	GTGGAATCT	AAAATACACA	AACAATTAGA	ATCAGTAGTT	3540
10	TAACACATTA	TACACTTAAA	AATTTTATAT	TTACCTTAGA	GCTTTAAATC	TCTGTAGGTA	3600
	GTTTGTCCAA	TTATGTCACA	CCACAGAAGT	AAGGTTCTT	CACAAAGATC	CGGGACCAAA	3660
	GCGGCCATCG	TGCCTCCCA	CTCCTGCAGT	TCGGGGGCAT	GGATGCGCGG	ATAGCCGCTG	3720
15	CTGGTTTCCT	GGATGCCGAC	GGATTTCAC	TGCCGGTAGA	ACTCCGCGAG	GTCGTCCAGC	3780
	CTCAGGCAGC	AGCTGAACCA	ACTCGCGAGG	GGATCGAGCC	CGGGGTGGGC	GAAGAACTCC	3840
	AGCATGAGAT	CCCCGCGCTG	GAGGATCATC	CAGCCGGCGT	CCCGGAAAAC	GATTCCGAAG	3900
20	CCCAACCTTT	CATAGAAGGC	GGCGGTGGAA	TCGAAATCTC	GTGATGGCAG	GTTGGGCGTC	3960
	GCTTGGTCGG	TCATTTCGAA	CCCCAGAGTC	CCGCTCAGAA	GAACTCGTCA	AGAAGGCGAT	4020
	AGAAGGCGAT	GCGCTCGGAA	TCGGGAGCGG	CGATACCGTA	AAGCACGAGG	AAGCGGTCAG	4080
	CCCATTCGCC	GCCAAGCTCT	TCAGCAATAT	CACGGGTAGC	CAACGCTATG	TCCTGATAGC	4140
25	GGTCCGCCAC	ACCCAGCCGG	CCACAGTCGA	TGAATCCAGA	AAAGCGGCCA	TTTTCCACCA	4200
	TGATATTCCG	CAAGCAGGCA	TCGCCATGGG	TCACGACGAG	ATCCTCGCCG	TCGGGCATGC	4260
	GCGCCTTGAG	CCTGGCGAAC	AGTTCGGCTG	GCGCGAGCCC	CTGATGCTCT	TCGTCCAGAT	4320
30	CATCCTGATC	GACAAGACCG	GCTTCCATCC	GAGTACGTGC	TCGCTCGATG	CGATGTTTCG	4380
	CTTGGTGGTC	GAATGGGCG	GTAGCCGGAT	CAAGCGTATG	CAGCCGCCGC	ATTGCATCAG	4440
	CCATGATGGA	TACTTTCTCG	GCAGGAGCAA	GGTGAGATGA	CAGGAGATCC	TGCCCCGGCA	4500
35	CTTCGCCCAA	TAGCAGCCAG	TCCCTTCCCG	CTTCAGTGAC	AACGTCGAGC	ACAGCTGCGC	4560
	AAGGAACGCC	CGTCGTGGCC	AGCCACGATA	GCCGCGCTGC	CTCGTCTTGC	AGTTCATPCA	4620
	GGGCACCGGA	CAGGTCGGTC	TTGACAAAAA	GAACCGGGCG	CCCCTGCGCT	GACAGCCGGA	4680
	ACACGGCGGC	ATCAGAGCAG	CCGATTGTCT	GTGTGCCCCA	GTCATAGCCG	AATAGCCTCT	4740
40	CCACCCAAGC	GGCCGGAGAA	CCTGCGTGCA	ATCCATCTTG	TTCAATCATG	CGAAACGATC	4800
	CTCATCCTGT	CTCTTGATCA	GATCTTGATC	CCCTGCGCCA	TCAGATCCTT	GGCGGCAAGA	4860
	AAGCCATCCA	GTTTACTTTG	CAGGGCTTCC	CAACCTTACC	AGAGGGCGCC	CCAGCTGGCA	4920
45	ATTCGGGTTC	GCTTGCTGTC	CATAAAACCG	CCCAGTCTAG	CTATCGCCAT	GTAAGCCCAC	4980
	TGCAAGCTAC	CTGCTTTCTC	TTTGCGCTTG	CGTTTCCCT	TGTCCAGATA	GCCCAGTAGC	5040
	TGACATTCAT	CGGGGTCAG	CACCGTTTCT	GCGGACTGGC	TTTCTACGTG	TTCCGCTTCC	5100
50	TTTAGCAGCC	CTGCGCCCT	GAGTGCTTGC	GGCAGCGTGA	AGCTAGCTTT	TTGCAAAAGC	5160
	CTAGGCCCTCC	AAAAAAGCCT	CCTCACTACT	TCTGGAATAG	CTCAGAGGCC	GAGGCGGCCT	5220

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	CGGCCTCTGC ATAAATAAAA AAAATTAGTC AGCCATGGGG CGGAGAATGG GGCGGGATGG	5280
	GCGGAGTTAG GGGGAACTG GGGGAGTTA GGGGCGGGAC TATGGTTGCT GACTAATTGA	5340
10	GATGCATGCT TTGCATACTT CTGCCTGCTG GGGAGCCTGG GGACTTTCCA CACCTGGTTG	5400
	CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC TGGGGAGCCT GGGGACTTTC	5460
	CACACCCTAA CTGACACACA TTCCACAGCT GCCTCGCGCG TTTCGGTGAT GACGGTGAAA	5520
15	ACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTTG TCTGTAAGCG GATGCCGGGA	5580
	GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC GCAGCCATGA	5640
	CCGAGTCACG TAGCGATAGC GGAGTGTATA CTGGCTTAAC TATGCGGCAT CAGAGCAGAT	5700
20	TGTA CTGAGA GTGCACCATA TGCGGTGTGA AATACCGCAC AGATGCGTAA GGAGAAAATA	5760
	CCGCATCAGG CGCTCTTCGG CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCCGGT	5820
	GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG TTATCCACAG AATCAGGGGA	5880
	TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC	5940
25	CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG	6000
	CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG	6060
	AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT	6120
30	TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCCGGT	6180
	GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCGGTTACGC CCGACCGCTG	6240
	CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT	6300
35	GGCAGCAGCC ACTGGAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT	6360
	CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA TCTGCGCTCT	6420
	GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC	6480
	CGCTGCTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGGCAGAA AAAAAGGATC	6540
40	TCAAGAAGAT CCTTTGATCT TTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCAGC	6600
	TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA	6660
	AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA	6720
45	ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTTCAT CCATAGTTGC	6780
	CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC	6840
	TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC	6900
50	AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT	6960
	TAATTGTTGC CGGGAAGCTA GAGTAAAGT TTCGCCAGTT AATAGTTTGC GCAACGTTGT	7020

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	TGCCATTGCT GCAGGCATCG TGGTGTACAG CTCGTGCTTT GGTATGGCTT CATTGAGCTC	7080
	CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCATG TTGTGCAAAA AAGCGGTTAG	7140
	CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTAT CACTCATGGT	7200
	TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC	7260
	TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCCACCGA GTTGCTCTTG	7320
	CCCCGGCTCA ACACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT	7380
	TGAAAAACGT TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC	7440
	GATGTAACCC ACTCGTGAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC	7500
	TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA	7560
	ATGTTGAATA CTCATACTCT TCCTTTTTC AATATTATTGA AGCATTTATC AGGGTTATTG	7620
	TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCGCGC	7680
	CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC	7740
	CTATAAAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTTCAA GAATTCATCG ATATCGGAAA	7800
	ATGAAAAAAA ATATTTTTTA ATTTTAAAAAT GAAATGTTTA TTTTCAATTT CTCCAAATTT	7860
	CACAAGGAAA GATTAGTCAC GGGTATGGGA GAGCAGAGGA CCATAAGAGT TCAGGAATAG	7920
	AATCCATTAT GATTCTGGAG TCAAGGAAGT ACTGATGCCA AGGTTTCAGT ATAAGAGCAG	7980
	TATCCACTGG AAAGGATAAA GTCACTACAA CTGAGCACAG AGCAGGACAG CTACCTAATG	8040
	AGTGGTCACT AATGGGCCAC TGTTACTACTG TTATACGGCT TAGGAATGAG CACTGAGGCT	8100
	GTGAGGTGTA TGGGTAAGGA CATCAGGATG TAAACCCAGC TCAGGTAGAG GACTCAGAGC	8160
	ACAGCACAAT CAGCACGAAC TAATAAACAA CAGATAAGAT AAGGCACAAG CTCAGCAATA	8220
	TTGGATCAGG GATCTTTGTA AATCTGACTG TGTATTCACT CTAGTTCAAT GTGACTCATG	8280
	AAGCCCCACC ATATGCAAAT CTAGAGAAGA CTITAGAGTA TAAATCTGAG GCTCACCTCA	8340
	CATACCAGCA AGGGAGTGAC CAGCTTGCTT TAAGGCACCA CTGAGCCCAA GTCTTAGACA	8400
	TCATGGATTG GCTGTGGAAC TTGCTATTCC TGATGGCAGC TGCCCAAGGT AAGTCATCAG	8460
	AAAAAAGAGT TCCAAGGGAA ATTGAAGCAG TTCCGAGCTC GGTACCCTCG AGATCCTAGA	8520
	GAGGTCTGGT GGAGCCTGCA AAAGTCCAGC TTTCAAAGGA ACACAGAAGT ATGTGTATGG	8580
	AATATTAGAA GATGTTGCTT TTAATCTTAA GTTGGTTCCT AGGAAAAATA GTTAAATACT	8640
	GTGACTTTAA AATGTGAGAG GGTTTTCAAG TACTCATTTT TTTAAATGTC CAAAATTTTT	8700
	GTCAATCAAT TTGAGGTCCT GTTTGTGTAG AACTGACATT ACTTAAAGTT TAACCGAGGA	8760
	ATGGGAGTGA GGCTCTCTCA TACCCTATCC AGAACTGACT TTTAACAATA ATAAATTAAG	8820

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TTTAAAAATAT TTTTAAATGA ATTGAGCAAT GTTGAGTTGA GTCAAGATGG CCGATCAGAA 8880  
 CCAGAACACC TGCAGCAGCT GGCAGGAAGC AGGTCATGTG GCAAGGCTAT TTGGGGAAGG 8940  
 10 GAAAAATAAAA CCACTAGGTA AACCTGTAGC TGTGGTTTGA AGAAGTGGTT TTGAAACACT 9000  
 CTGTCCAGCC CCACCAAACC GAAAGTCCAG GCTGAGCAAA ACACCACCTG GGTAATTTGC 9060  
 ATTTCTAAAA TAAGTTGAGG ATTCAGCCGA AACTGGAGAG GTCCTCTTTT AACTTATTGA 9120  
 15 GTTCAACCTT TTAATTTTAG CTTGAGTAGT TCTAGTTTCC CCAAACCTAA GTTTATCGAC 9180  
 TTCTAAAAATG TATTTAGAAT T 9201

## (2) INFORMATION FOR SEQ ID NO:10:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 7059 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGATCCAGAC ATGATAAGAT ACATTGATGA GTTTGGACAA ACCACAACCTA GAATGCAGTG 60  
 30 AAAAAAATGC TTTATTTGTG AAAATTGTGA TGCTATGTCT TTATTTGTAA CCATTATAAG 120  
 CTGCAATAAA CAAGTTAACA ACAACAATTG CATTCAATTTT ATGTTTCAGG TTCAGGGGGA 180  
 GGTGTGGGAG GTTTTTTAAA GCAAGTAAAA CCTCTACAAA TGTGGTATGG CTGATTATGA 240  
 35 TCTCTAGTCA AGGCACTATA CATCAAATAT TCCTTATTAA CCCCTTACA AATTAAAAAG 300  
 CTAAAGGTAC ACAATTTTTG AGCATAGTTA TTAATAGCAG AACTCTATG CCTGTGTGGA 360  
 GTAAGAAAAA ACAGTATGTT ATGATTATAA CTGTTATGCC TACTTATAAA GGTTACAGAA 420  
 TATTTTTCCTA TAATTTTCTT GTATAGCAGT GCAGCTTTTT CCTTTGTGGT GTAAATAGCA 480  
 40 AAGCAAGCAA GAGTTCTATT ACTAAACACA GCATGACTCA AAAAACTTAG CAATTCTGAA 540  
 GGAAAGTCCT TGGGGTCTTC TACCTTTCTC TTCTTTTTTG GAGGAGTAGA ATGTTGAGAG 600  
 TCAGCAGTAG CCTCATCATC ACTAGATGGC ATTTCTTCTG AGCAAAACAG GTTTTCCTCA 660  
 45 TTAAAGGCAT TCCACCACTG CTCCCATTC ACGTTCCAT AGGTTGGAAT CTAAAAATACA 720  
 CAAACAATTA GAATCAGTAG TTTAACACAT TATACACTTA AAAATTTTAT ATTTACCTTA 780  
 GAGCTPTAAA TCTCTGTAGG TAGTTTGTCC AATTATGTCA CACCACAGAA GTAAGGTTCC 840  
 50 TTCACAAAGA TCCGGGGCCC ACTCATAAAT CCAGTTGCCG CCACGGTAGC CAATCACCGT 900  
 ATCGTATAAA TCATCGTCGG TACGTTCCGC ATCGCTCATC ACAATACGTG CCTGGACGTC 960

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	GAGGATTTTCG	CGTGGGTCAA	TGCCGCGCCA	GATCCACATC	AGACGGTTAA	TCATGCGATA	1020
	CCAGTGAGGG	ATGGTTTAC	CATCAAGGGC	CGACTGCACA	GGCGGTGTG	CGCCGTGATT	1080
10	AAAGCGGCGG	ACTAGCGTCG	AGGTTTCAGG	ATGTTTAAAG	CGGGGTTGA	ACAGGGTTTC	1140
	GCTCAGGTTT	GCCTGTGTCA	TGGATGCAGC	CTCCAGAATA	CTTACTGGAA	ACTATTGTAA	1200
	CCCGCTGAA	GTTAAAAAGA	ACAACGCCG	GCAGTGCCAG	GCGTTGAAAA	GATTAGCGAC	1260
15	CGGAGATTGG	CGGGACGAAT	ACGACGCCA	TATCCCACGG	CTGTTCAATC	CAGGTATCTT	1320
	GCGGGATATC	AACAACATAG	TCATCAACCA	GCGGACGACC	AGCCGGTTTT	GCGAAGATGG	1380
	TGACAAAGTG	CGCTTTTGA	TACATTTCAC	GAATCGCAAC	CGCAGTACCA	CCGGTATCCA	1440
20	CCAGGTCATC	AATAACGATG	AAGCCTTCGC	CATCGCCTTC	TGCGCGTTTC	AGCACTTTAA	1500
	GCTCGCGCTG	GTTGTCTGA	TCGTAGCTGG	AAATACAAAC	GGTATCGACA	TGACGAATAC	1560
	CCAGTTCACG	CGCCAGTAAC	GCACCCGGTA	CCAGACCGCC	ACGGCTTACG	GCAATAATGC	1620
	CTTTCCATTG	TCAGAAAGGC	ATCAGTCGGC	TTGCGAGTTT	ACGTGCATGG	ATCTGCAACA	1680
25	TGTCCACGGT	GACGATGTAT	TTTTCGCTCA	TGTGAAGTGT	CCCAGCCTGT	TTATCTACGG	1740
	CTTAAAAAGT	GTTTCGAGGGG	AAAATAGGTT	GCGCGAGATT	ATAGAGATCA	GCTTTTTGCA	1800
	AAAGCCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	AGGCCGAGGC	1860
30	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAT	TAGTCAGCCA	TGGGGCGGAG	AATGGGGCGG	1920
	GATGGGCGGA	GTTAGGGCGG	AACTGGGCGG	AGTTAGGGGC	GGGACTATGG	TTGCTGACTA	1980
	ATTGAGATGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	2040
35	CGCTCTTCGG	CTTCCTCGCT	CACCTGACTCG	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	2100
	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	2160
	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	2220
	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	2280
40	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	2340
	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCTTT	TCTCCCTTCG	2400
	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	2460
45	CGTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	2520
	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	2580
	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	2640
50	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	2700
	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	2760

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	GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT	2820
	CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGAACG AAAACTCACG TTAAGGGATT	2880
10	TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA AAAATGAAGT	2940
	TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC	3000
	AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCTTCAT CCATAGTTGC CTGACTCCCC	3060
15	GTCTGTGTA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA	3120
	CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG	3180
	GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC	3240
20	CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT	3300
	ACAGGCATCG TGGTGTACG CTCGTCGTTT GGTATGGCTT CATTGAGCTC CGGTTCCCAA	3360
	CGATCAAGGC GAGTTACATG ATCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT	3420
	CCTCCGATCG TTGTGAGAAG TAAGTTGGCC GCAGTGTAT CACTCATGGT TATGGCAGCA	3480
25	CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC	3540
	TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA	3600
	ATACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT	3660
30	TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC	3720
	ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA	3780
	AAAACAGGAA GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA	3840
35	CTCATACTCT TCCTTTTTC AATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC	3900
	GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTC	3960
	CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAACT CTATAAAAAAT	4020
	AGGCGTATCA CGAGGCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA	4080
40	CACATGCAGC TCCCGGAGAC GGTACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA	4140
	GCCCGTCAGG GCGCGTCAGC GGGTGTGGC GGGTGTGGG GCTGGCTTAA CTATGCGGCA	4200
	TCAGAGCAGA TTGTACTGAG AGTGCACCAT AGCGGCCGCA TATGCGGTGT GAAATACCGC	4260
45	ACAGATGCGT AAGGAGAAAA TACCGCATCA GGCGCCATTC GCCATTGAGG CTGCGCAACT	4320
	GTTGGGAAGG GCGATCGGTG CGGGCCTCTT CGCTATTACG CCAGAAATCG GCCCAGGGGA	4380
	CTGTGAGGAC AGAAGGCTTG TGGGTTTGAG GGAGGACTGT CTTGCAGAGG ATGATAGGGT	4440
50	AAAATAGAAT GAAGGATGAT TTTTATAAAT GGTACGTGC CTTAGGATGA CTACATATTT	4500
	AGTCCCTTAT AAGAGAAATT GAGTAGTTGG TAAAACAACA GATAATAATT ATTAAATGAG	4560

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	GAAAGAGAGA	AACCACAGGT	GCAAAGATTG	ACTTTATTTA	TTCATTCTCC	TCCAACATTA	4620
	GCATAATTAA	AGCCAAGGAG	GAGGAGGGGG	GTGAGGTGAA	AGATGAGCTG	GAGGACCGCA	4680
10	ATAGGGGTAG	GTCCCTGTG	GAAAAAGGGT	CAGAGGCCAA	AGGATGGGAG	GGGGTCAGGC	4740
	TGGAACGAG	GAGCAGGTGG	GGGCACCTCT	CCCTCTAACA	CTCTCCCCTG	TTGAAGCTCT	4800
	TTGTGACGGG	CGAGCTCAGG	CCCTGATGGG	TGACTTCGCA	GGCGTAGACT	TTGTGTTTCT	4860
15	CGTAGTCTGC	TTTGCTCAGC	GTCAGGGTGC	TGCTGAGGCT	GTAGGTGCTG	TCCTTGCTCT	4920
	CCTGCTCTGT	GACACTCTCC	TGGGAGTTAC	CCGATTGGAG	GGCGTTATCC	ACCTTCCACT	4980
	GTACTTTGGC	CTCTCTGGGA	TAGAAGTTAT	TCAGCAGGCA	CACAACAGAG	GCAGTTCCAG	5040
20	ATTTCAACTG	CTCATCAGAT	GGCGGAAGA	TGAAGACAGA	TGGTGACGCC	ACAGTTCCCTG	5100
	AGGAAAGAAG	CAAACAGGAT	GGTGTTTAAG	TAACAAAGTT	CTGCCCTTGG	GTGTGTGTGT	5160
	TGCGGATAAG	GGCATGTTAG	GGACAGACAG	AAAACAGCAT	GCTTATCCCA	GATAATTATA	5220
	GCAAGGAGAC	CAAGAAGCGT	ATTTAAAAATC	TTGATGTTTT	GAGTTTCTTC	CTAGCTTCCC	5280
25	CCTATTCCCTT	AATAAAGTTC	TAAATGTGTT	TGTTGGAGCT	CTTTCAGGCC	ATTCTGAGGG	5340
	CTTTCGATGC	TTTTCTGACC	TTGCAGTAAA	CTCAATGCTT	TAGGCAAAGA	ATGGCCACGT	5400
	CATCCGACCC	CCTCAGAGTT	TAGAATTCAT	CGATATCTAG	ATCCTAGATA	ATTGCATTCA	5460
30	TTTAAAAAAA	AAATATTTCT	CCTAAAATGA	ATACTCAGAA	AGTGGTCTTG	AAAAAGATTT	5520
	GTGAAGCCGT	TTTGACCAGA	ATGTCAAAGT	CTTAATAGTA	AGGCAAAACA	AACAACATAA	5580
	AAAGATCATG	AACAAAGTCA	CTGTAAAGAC	TTCGGGTATT	GGAAAAAAT	TGAATGGAGA	5640
35	CCAATAATCA	GAGGGAAGAA	TAATAGAGTA	ATTTTAAGAA	GTTTTCTAAA	TATATTAGAA	5700
	ATTAAAGACA	CTAAAGTCCT	TCAATTTCTT	ACATAACCTA	ATTTTGAAAA	TGAATTCATA	5760
	ATACATTTTA	GAAGTCGATA	AACTTAAGTT	TGGGGAAACT	AGAACTACTC	AAGCTAAAAAT	5820
	TAAAAGGTTG	AACTCAATAA	GTTAAAAGAG	GACCTCTCCA	GTTTCGGCTG	AATCCTCAAC	5880
40	TTATTTTAGA	AATGCAAATT	ACCCAGGTGG	TGTTTTGCTC	AGCCTGGACT	TTCGGTTTGG	5940
	TGGGGCTGGA	CAGAGTGTTC	CAAAACCACT	TCTTCAAACC	ACAGCTACAA	GTTTACCTAG	6000
	TGGTTTTATT	TTCCCTTCCC	CAAATAGCCT	TGCCACATGA	CCTGCTTCCT	GCCAGCTGCT	6060
45	GCAGGTGTTT	TGGTTCTGAT	CGGCCATCTT	GACTCAACTC	AACATTGCTC	AATTCATTTA	6120
	AAAATATTTT	AAACTTAATT	TATTATTGTT	AAAAGTCAGT	TCTGGATAGG	GTATGAGAGA	6180
	GCCTCACTCC	CATTCCCTCG	TTAAACTTTA	AGTAATGTCA	GTTCTACACA	AACAAGACCT	6240
50	CAAAATGATT	GACAAAAATT	TTGGACATTT	AAAAAAATGA	GTAATTGAAA	ACCCCTCTCAC	6300
	ATTTTAAAGT	CACAGTATTT	AACTATTTTT	CCTAGGAACC	AACTTAAGAG	TAAAAGCAAC	6360

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ATCTTCTAAT ATTCCATACA CATACTTCTG TGTTCCCTTTG AAAGCTGGAC TTTTGCAGGC 6420  
 TCCACCAGAC CTCTCTAGGA TCTCGAGCTC GCGAAAGCTT GCATGCCTGC AGGTCGACTC 6480  
 10 TAGAGGATCA AACCAACTGT CTTTGAGTAG AGCCAAAATT GTTGATATAC TTTGAATTTT 6540  
 AATTATATTT CTTGCTGAGC AGAGGTGGCA AGAGTTTTC AATATGTGCA AAACCACCTC 6600  
 ATGTTCCCTT CACCTGGGAG CCAGAGTAGC AGGAGGAAGA GAAGCTGAGC TGGGGCTTCC 6660  
 15 ATGTTCCCTT CTGGGTCCTA ACTGAGCAGT TCCTCCCCAG GGCTCTGACA CAGGCATTGA 6720  
 TATGGGCTCT GGAAGGTAGG GCAGCTGGGA GGGACATGCA AAGCAGCTGG GTGGGAGCTG 6780  
 AGCTTCCAGC TGCAGAGACC ACCTGCTTCT TCCTCTCTGC ACTGAGCATC CTGCGCCACC 6840  
 CTGGTTGTCA GGCCAGAAAA GTCTGTTGGC TCAGTCTGAG TGTAGAACTT CTCCCTGTG 6900  
 20 CTCAGAGAAT TTCATTCTTA TGTCTTTCTT CTCCTCAATC ACCTAAATTC ACCCAGATGA 6960  
 TGTTTGGCAC AAGCCTGTTA AGAACAATAT AAAAGGCTGT GTTTTCATTT CTCTCTTCCT 7020  
 ATCCTCAATA TGCCAGTCA TCTCCCTAAG TGCATTATT 7059

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(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 81 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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AAAAAAGAAAT TCGAGCTCTT CTGATAACGC TGTCCTTCTG TTTGCAGGTG TCCAGTGTCA 60  
 GGTCCAACCTT GTCCAGTCCG G 81

(2) INFORMATION FOR SEQ ID NO:12:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 93 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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TTAACCTTCGG CACCGGACTG GACAAGTTGG ACCTGACACT GGACACCTGC AAACAGAAGG 60  
 ACAGCGTTAT CAGAAGAGCT CGAATTCCTT TTT 93

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## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGCCGAAGTT AAGAAGCCTG GCGCTTCTGT GAAGGTCTCC TGCAAGGCTT CTGGCTACAC 60  
 CTTACCCGAC TACTGGATGA ACTGGGTTCC 90

20

## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCAGGTGCCT GTCGAACCCA GTTCATCCAG TAGTCGGTGA AGGTGTAGCC AGAAGCCTTG 60  
 CAGGAGACCT TCACAGAAGC GCCAGGCTTC 90

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## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ACAGGCACCT GGACAGGGCC TAGAGTGGAT GGGAAGGATT GATCCTTCCG ATAGTGAAAC 60  
 TCACTACAAT CAGAAGTTCC AGGGTAGGGT 90

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## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGGGTCATTG TTACCCTACC CTGGAAC TTC TGATTGTAGT GAGTTTCACT ATCGGAAGGA 60

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TCAATCCTTC CCATCCACTC TAGGCCCTGT 90

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 90 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AACAATGACC CGAGACACAT CCACCAGCAC AGTCTACATG GAACTCAGCA GCCTGCGATC 60

TGAGGACACC GCAGTCTATT ACTGTGCACG 90

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(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 90 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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AGCCGTCCAC CTCGTGCACA GTAATAGACT GCGGTGTCCT CAGATCGCAG GCTGCTGAGT 60

TCCATGTAGA CTGTGCTGGT GGATGTGTCT 90

(2) INFORMATION FOR SEQ ID NO:19:

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(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 93 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGGTGGACGG CTCGGTTCCT TTGCTATGGA CTACTGGGGT CAAGGCACCC TCGTCACCGT 60  
 CTCCTCAGGT GAGTCCTCAC ACTCGAGGTC GAC 93

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAAAAAGTCG ACCTCGAGTG TGAGGACTCA CCTGAGGAGA CCGTGACGAG GGTGCCTTGA 60  
 CCCCAAGTAGT CCATAGCAAA GGAACCG 87

25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTTTTTGAAT TCAAGCTTTC CTGACTACAT GAGTGCATTT CTGTTTTATT TCCAATTTC 60  
 GATACCACCG GAGAAATTGT GCTAACACAA 90

35

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 78 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)

40

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATTTCTCCG GTGGTATCTG AAATTGGAAA TAAAACAGAA ATGCACTCAT GTAGTCAGGA 60  
 AAGCTTGAAT TCAAAAAA 78

50

55

5

## (2) INFORMATION FOR SEQ ID NO:23:

- 10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCTCCAGCTA CATTGTCTTT GTCTCCAGGT GAGAGAGCCA CTCTATCCTG CAGAGCCAGT 60  
 GAAAGTGTG ATAGTTATGG CAATAGT 87

20

## (2) INFORMATION FOR SEQ ID NO:24:

- 25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ACTATCAACA CTTTCACTGG CTCTGCAGGA TAGAGTGGCT CTCTCACCTG GAGACAAAGA 60  
 CAATGTAGCT GGAGATTGTG TTAGCAC 87

35

## (2) INFORMATION FOR SEQ ID NO:25:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

45 TTTATGCACT GGTACCAGCA GAAACCAGGA CAGGCACCAA GGCTCCTCAT CTATCGTGCA 60  
 TCCAACCTAG AAACGTGTAT CCCTGCC 87

## (2) INFORMATION FOR SEQ ID NO:26:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 base pairs  
 (B) TYPE: nucleic acid

55

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AGTTTCTAGG TTGGATGCAC GATAGATGAG GAGCCTTGGT GCCTGTCCTG GTTTCCTGCTG 60

15

GTACCAGTGC ATAAACTAT TGCCATA 87

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: DNA (genomic)

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGGTTTCAGTG GCAGTGGTTC TAGGACAGAC TTCACTCTCA CCTATTCTTC TCTAGAGCCT 60

GAAGATTTTG CAGTGTATTA CTGTCAG 87

30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

40

CACTGCAAAA TCTTCAGGCT CTAGAGAAGA ATAGGTGAGA GTGAAGTCTG TCCTAGAACC 60

ACTGCCACTG AACCTGGCAG GGATACC 87

(2) INFORMATION FOR SEQ ID NO:29:

45

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CAAAGTAATG AGGATCCTCG GACGTTCCGGT GGAGGCACCA AGGTGGAAGA GAAACGTAAG 60  
TGCACTTTCC TCGAGGTCGA CTTTTTT 87

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 99 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAAAAGTCG ACCTCGAGGA AAGTGCACCTT ACGTTTCTCT TCCACCTTGG TGCCTCCACC 60  
GAACGTCCGA GGATCCTCAT TACTTTGCTG ACAGTAATA 99

25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAAAAAGAAT TCGAGCTCTT TTCTGATAAC GTTGTCTTTC TGTTCCTTGC AGGTGTCCAG 60  
TGTCAGGTCC AACTTCAGCA GCCTGGG 87

35

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)  
(iv) ANTI-SENSE: YES

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45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAAAAAGTCG ACTGTGAGGA CTCACCTGAG GAGACGGTGA CTGAGGTGCC T 51

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(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAAAAAGAAT TCAAGCTTTC CTGACTACAT GAGTGCATTT CTGTTTATT TCCAATTCA 60  
 GATACCACCG GAGACATTGT GCTAACACAA TCTCCA 96

20

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 54 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iv) ANTI-SENSE: YES

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30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAAAAAGTCG ACCTCGAGAT CACTTACGTT TGATTTCAG CTGGTGCCT CCAC 54

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## Claims

- 5 1. A method for producing a humanized monoclonal antibody by utilizing a process of comparative model building comprising:
  - a) selecting a monoclonal antibody to be humanized;
  - b) searching computer databanks for protein crystal structures that demonstrate greater than 50 percent sequence homology to the variable region of said antibody to produce a structural template;
  - 10 c) determining the structure of the complementarity determining region, or CDR, loops and assigning the loops to canonical loop conformations;
  - d) determining the framework residues which are crucial to the conformation of the CDR loops;
  - e) replacing the CDR loops of the structural templates with canonical CDR backbone templates using interactive computer graphics;
  - 15 f) searching computer databanks to extract initial backbone approximations for each loop for non-canonical CDR loops;
  - g) replacing all non-conserved amino-acid side chains in similar positions on said antibody and on the computer model with human amino acid residues using interactive computer graphics to produce a model having a combination of backbone fragments of different antibodies with replaced side chains;
  - 20 h) solvating the models with a water layer corresponding to about 7 angstroms;
  - i) refining the structure with an energy minimization protocol to produce a structure wherein all atoms of the system are freely mobile;
  - j) searching computer databanks to find homologous human sequences for the variable light and variable heavy chains of the antibody;
  - 25 k) combining the sequences found in (j) to obtain human templates;
  - l) comparing the structural template of (a) with the human templates of (k) and selecting a human template with variable regions having greater than 50 percent sequence identity with the structural template;
  - m) determining the CDR loops of the human template selected in (l);
  - n) replacing the CDR loop region of the selected human template with the analogous sequences from the antibody to produce a Phase 1 humanized sequence;
  - 30 o) superimposing the models of the antibody and the Phase 1 humanized sequence to compare the binding site regions;
  - p) identifying by the comparison in (o) all amino acids in the framework residues and CDR junction residues that interact with the antibody CDR loops that can be important to the structural integrity of the antibody binding site;
  - 35 q) reinserting into the Phase 1 humanized sequence all amino acid residues identified in (p) to be different from those in the antibody, and refining the resultant structure with an energy minimization protocol to produce a Phase II humanized sequence;
  - r) refining the Phase II humanized sequence using iterative conformational search protocols on all regions of the binding site and by analysis of the binding site to determine which regions of the CDR surface or residues at the CDR-framework junction are not likely to involve antigen binding; and
  - 40 s) replacing the amino acids in the non-antigen binding regions of the binding site with amino acid residues corresponding to the human residues to produce a humanized monoclonal antibody.
2. The method of Claim 1 wherein the monoclonal antibody is a murine antibody.
- 45 3. The method of Claim 2 wherein the monoclonal antibody is an anti-CD18 monoclonal antibody.
4. The method of Claim 3 wherein the monoclonal antibody is 60.3
- 50 5. A humanized monoclonal antibody having the structural and binding characteristics of the anti-CD18 monoclonal antibody 60.3
6. The humanized monoclonal antibody of Claim 5 wherein the amino acid at position 50 in Figure 2 is changed from Arg to Asp.
- 55 7. The humanized monoclonal antibody of Claim 6, wherein the amino acid at position 54 is changed from Leu to Arg and the amino acid at position 55 is changed from Glu to Ala.
8. The humanized monoclonal antibody of Claim 7 wherein the amino acid at position 68 is changed from Arg to Gly.

## 60.3 Heavy Chain Sequences

Kabat #	hVh/ Jh4	21-2 CL	h60.3 template	h60.3 Phase IV	h60.3 Phase II/III	h60.3 Phase I	m60.3	
1	Gln	Gln	Gln	Gln	Gln	Gln	Gln	
2	Val	Val	Val	Val	Val	Val	Val	
3	Gln	Gln	Gln	Gln	Gln	Gln	Gln	
4	Leu	Leu	Leu	Leu	Leu	Leu	Leu	
5	Val	Val	Val	Val	Val	Val	Gln	
6	Gln	Gln	Gln	Gln	Gln	Gln	Gln	
7	Ser	Ser	Ser	Ser	Ser	Ser	Pro	
8	Gly	Gly	Gly	Gly	Gly	Gly	Gly	
9	Ala	Ala	Ala	Ala	Ala	Ala	Pro	
10	Glu	Glu	Glu	Glu	Glu	Glu	Asp	
11	Val	Val	Val	Val	Val	Val	Leu	
12	Lys	Lys	Lys	Lys	Lys	Lys	Leu	
13	Lys	Lys	Lys	Lys	Lys	Lys	Lys	
14	Pro	Pro	Pro	Pro	Pro	Pro	Pro	
15	Gly	Gly	Gly	Gly	Gly	Gly	Gly	FR 1
16	Ala	Ala	Ala	Ala	Ala	Ala	Ala	
17	Ser	Ser	Ser	Ser	Ser	Ser	Pro	
18	Val	Val	Val	Val	Val	Val	Val	
19	Lys	Lys	Lys	Lys	Lys	Lys	Lys	
20	Val	Val	Val	Val	Val	Val	Leu	
21	Ser	Ser	Ser	Ser	Ser	Ser	Ser	
22	Cys	Cys	Cys	Cys	Cys	Cys	Cys	
23	Lys	Lys	Lys	Lys	Lys	Lys	Lys	
24	Ala	Ala	Ala	Ala	Ala	Ala	Ala	
25	Ser	Ser	Ser	Ser	Ser	Ser	Ser	
26	Gly	Gly	Gly	Gly	Gly	Gly	Gly	*H1
27	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	*H1
28	Thr	Thr	Thr	Thr	Thr	Thr	Thr	
29	Phe	Phe	Phe	Phe	Phe	Phe	Phe	*H1
30	Thr	Thr	Thr	Thr	Thr	Thr	Thr	H1
31	Ser	Ser	Asn	Asn	Asp	Asp	Asp	
32	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	
33	Ala	Tyr	Tyr	Tyr	Trp	Tyr	Trp	
34	Leu	Met	Met	Met	Met	Met	Met	*H1
35	Ser	His	His	His	Asn	His	Asn	
36	Trp	Trp	Trp	Trp	Trp	Trp	Trp	
37	Val	Val	Val	Val	Val	Val	Val	
38	Arg	Arg	Arg	Arg	Arg	Arg	Lys	
39	Gln	Gln	Gln	Gln	Gln	Gln	Gln	
40	Ala	Ala	Ala	Ala	Ala	Ala	Arg	
41	Pro	Pro	Pro	Pro	Pro	Pro	Pro	FR 2
42	Gly	Gly	Gly	Gly	Gly	Gly	Gly	
43	Gln	Gln	Gln	Gln	Gln	Gln	Arg	
44	Gly	Gly	Gly	Gly	Gly	Gly	Gly	

FIGURE 1 1/3



## 60.3 Heavy Chain Sequences

Kabat #	NVH/Jh4	21-2 CL	h60.3 template	h60.3 Phase IV	h60.3 Phase II/III	h60.3 Phase I	m60.3
45	Leu	Leu	Leu	Leu	Leu	Leu	Leu
46	Glu	Glu	Glu	Glu	Glu	Glu	Glu
47	Trp	Trp	Trp	Trp	Trp	Trp	Trp
48	Met	Met	Met	Met	Met	Met	Ile
49	Gly	Gly	Gly	Gly	Gly	Gly	Gly
50	Trp	Ile	Ile	Arg	Arg	Ile	Arg
51	Ile	Ile	Ile	Ile	Ile	Ile	Ile
52	Asn	Asn	Asn	Asp	Asp	Asp	Asp
52a	Pro	Pro	Pro	Pro	Pro	Pro	Pro
53	Gly	Ser	Ser	Ser	Ser	Ser	Ser
54	Asn	Gly	Gly	Asp	Asp	Asp	Asp
55	Gly	Gly	Asn	Ser	Ser	Ser	Ser
56	Asp	Ser	Ser	Glu	Glu	Ser	Glu
57	Thr	Thr	Thr	Thr	Thr	Thr	Thr
58	Asn	Ser	Asn	His	His	Asn	His
59	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
60	Ala	Ala	Ala	Asn	Asn	Ala	Asn
61	Gln	Gln	Gln	Gln	Gln	Gln	Gln
62	Lys	Lys	Lys	Lys	Lys	Lys	Lys
63	Phe	Phe	Phe	Phe	Phe	Phe	Phe
64	Gln	Gln	Gln	Gln	Gln	Gln	Lys
65	Gly	Gly	Gly	Gly	Gly	Gly	Asp
66	Arg	Arg	Arg	Arg	Arg	Arg	Lys
67	Val	Val	Val	Val	Val	Val	Ala
68	Thr	Thr	Thr	Thr	Thr	Thr	Thr
69	Ile	Met	Met	Met	Met	Met	Leu
70	Thr	Thr	Thr	Thr	Thr	Thr	Thr
71	Ala	Arg	Arg	Val	Val	Val	Val
72	Asp	Asp	Asp	Asp	Asp	Asp	Asp
73	Thr	Thr	Thr	Thr	Thr	Thr	Lys
74	Ser	Ser	Ser	Ser	Ser	Ser	Ser
75	Thr	Thr	Thr	Thr	Thr	Thr	Ser
76	Ser	Ser	Ser	Ser	Ser	Ser	Ser
77	Thr	Thr	Thr	Thr	Thr	Thr	Thr
78	Ala	Val	Val	Val	Val	Val	Ala
79	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
80	Met	Met	Met	Met	Met	Met	Ile
81	Glu	Glu	Glu	Glu	Glu	Glu	Gln
82	Leu	Leu	Leu	Leu	Leu	Leu	Leu
82a	Ser	Ser	Ser	Ser	Ser	Ser	Ser
82b	Ser	Ser	Ser	Ser	Ser	Ser	Ser
82c	Leu	Leu	Leu	Leu	Leu	Leu	Leu
83	Arg	Arg	Arg	Arg	Arg	Arg	Thr
84	Ser	Ser	Ser	Ser	Ser	Ser	Ser

FIGURE 1 2/3

## 60.3 Heavy Chain Sequences

Kabat #	hVh/Jh4	21-2 'CL	h60.3 template	h60.3 Phase IV	h60.3 Phase II/III	h60.3 Phase I	m60.3
85	Glu	Glu	Glu	Glu	Glu	Glu	Glu
86	Asp	Asp	Asp	Asp	Asp	Asp	Asp
87	Thr	Thr	Thr	Thr	Thr	Thr	Ser
88	Ala	Ala	Ala	Ala	Ala	Ala	Ala
89	Val	Val	Val	Val	Val	Val	Val
90	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
91	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
92	Cys	Cys	Cys	Cys	Cys	Cys	Cys
93	Ala	Ala	Ala	Ala	Ala	Ala	Ala
94	Arg	Arg	Arg	Arg	Arg	Arg	Arg
95			Glu	Gly	Gly	Gly	Gly
96			Lys				
97			Leu				
98			Ala				
99			Thr				
100			Thr				
100a			Ile				
100b			Phe				
100c			Gly	Gly	Gly	Gly	Gly
100d			Val	Arg	Arg	Arg	Arg
100e			Leu	Leu	Leu	Leu	Leu
100f			Ile	Gly	Gly	Gly	Gly
100g			Ile	Ser	Ser	Ser	Ser
100h			Thr	Phe	Phe	Phe	Phe
100i	Tyr		Gly	Ala	Ala	Ala	Ala
100j	Phe		Met	Met	Met	Met	Met
101	Asp		Asp	Asp	Asp	Asp	Asp
102	Tyr		Tyr	Tyr	Tyr	Tyr	Tyr
103	Trp		Trp	Trp	Trp	Trp	Trp
104	Gly		Gly	Gly	Gly	Gly	Gly
105	Gln		Gln	Gln	Gln	Gln	Gln
106	Gly		Gly	Gly	Gly	Gly	Gly
107	Thr		Thr	Thr	Thr	Thr	Thr
108	Leu		Leu	Leu	Leu	Leu	Ser
109	Val		Val	Val	Val	Val	Val
110	Thr		Thr	Thr	Thr	Thr	Thr
111	Val		Val	Val	Val	Val	Val
112	Ser		Ser	Ser	Ser	Ser	Ser
113	Ser		Ser	Ser	Ser	Ser	Ser

\* The h60.3 heavy chain was made with the sequence shown in the Phase II/III column, with the following exception:  
H71: Arg instead of Val

FIGURE 1 3/3

## 60.3 Light Chain Sequences

Kabat #	HVKL/Jk	h60.3 template	h60.3 Phase IV	h60.3 Phase I/II	h60.3 Phase I	m60.3	
1	Glu	Glu	Glu	Glu	Glu	Asp	
2	Asp	Asp	Asp	Asp	Asp	Asp	L1
3	Val	Val	Val	Val	Val	Val	
4	Leu	Leu	Leu	Leu	Leu	Leu	
5	Thr	Thr	Thr	Thr	Thr	Thr	
6	Gln	Gln	Gln	Gln	Gln	Gln	
7	Ser	Ser	Ser	Ser	Ser	Ser	
8	Pro	Pro	Pro	Pro	Pro	Pro	
9	Gly	Ala	Ala	Ala	Ala	Ala	
10	Thr	Thr	Thr	Thr	Thr	Ser	FR 1
11	Leu	Leu	Leu	Leu	Leu	Leu	
12	Ser	Ser	Ser	Ser	Ser	Ala	
13	Leu	Leu	Leu	Leu	Leu	Val	
14	Ser	Ser	Ser	Ser	Ser	Ser	
15	Pro	Pro	Pro	Pro	Pro	Leu	
16	Gly	Gly	Gly	Gly	Gly	Gly	
17	Glu	Glu	Glu	Glu	Glu	Gln	
18	Arg	Arg	Arg	Arg	Arg	Arg	
19	Ala	Ala	Ala	Ala	Ala	Ala	
20	Thr	Thr	Thr	Thr	Thr	Thr	
21	Leu	Leu	Leu	Leu	Leu	Asp	
22	Ser	Ser	Ser	Ser	Ser	Ser	
23	Cys	Cys	Cys	Cys	Cys	Cys	
24		Arg	Arg	Arg	Arg	Arg	
25		Ala	Ala	Ala	Ala	Ala	L1
26		Ser	Ser	Ser	Ser	Ser	
27		Gln	Glu	Glu	Glu	Glu	
28		Ser	Ser	Ser	Ser	Ser	
29		Val	Val	Val	Val	Val	L1
30		Ser	Asp	Asp	Asp	Asp	
31		Ser	Ser	Ser	Ser	Ser	L1
31a			Tyr	Tyr	Tyr	Tyr	
31b			Gly	Gly	Gly	Gly	
31c			Asn	Asn	Asn	Asn	
31d			Ser	Ser	Ser	Ser	
32		Tyr	Phe	Phe	Phe	Phe	
33		Leu	Met	Met	Met	Met	L1
34		Ala	His	His	Ala	His	
35		Trp	Trp	Trp	Trp	Trp	
36		Tyr	Tyr	Tyr	Tyr	Tyr	
37		Gln	Gln	Gln	Gln	Gln	
38		Gln	Gln	Gln	Gln	Gln	
39		Lys	Lys	Lys	Lys	Lys	
40		Pro	Pro	Pro	Pro	Pro	
41		Gly	Gly	Gly	Gly	Gly	FR 2
42		Gln	Gln	Gln	Gln	Gln	
43		Ala	Ala	Ala	Ala	Pro	
44		Pro	Pro	Pro	Pro	Pro	
45		Arg	Arg	Arg	Arg	Lys	
46		Leu	Leu	Leu	Leu	Leu	

FIGURE 2 1/3

## 60.3 Light Chain Sequences

Kabat #	hVdII/ Jk	h60.3 template	h60.3 Phase IV	h60.3 Phase I/II	h60.3 Phase I	h60.3 en60.3	
47	Leu	Leu	Leu	Leu	Leu	Leu	
48	Ile	Ile	Ile	Ile	Ile	Ile	L2
49	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	
50		Asp	Asp	Arg	Arg	Arg	
51		Ala	Ala	Ala	Ala	Ala	L2
52		Ser	Ser	Ser	Ser	Ser	
53		Asn	Asn	Asn	Asn	Asn	
54		Arg	Arg	Leu	Arg	Leu	
55		Ala	Ala	Glu	Ala	Glu	
56		Thr	Thr	Thr	Thr	Ser	
57	Gly	Gly	Gly	Gly	Gly	Gly	
58	Ile	Ile	Ile	Ile	Ile	Ile	
59	Pro	Pro	Pro	Pro	Pro	Pro	
60	Asp	Ala	Ala	Ala	Ala	Ala	
61	Arg	Arg	Arg	Arg	Arg	Arg	
62	Phe	Phe	Phe	Phe	Phe	Phe	
63	Ser	Ser	Ser	Ser	Ser	Ser	
64	Gly	Gly	Gly	Gly	Gly	Gly	L2
65	Ser	Ser	Ser	Ser	Ser	Ser	
66	Gly	Gly	Gly	Gly	Gly	Gly	
67	Ser	Ser	Ser	Ser	Ser	Ser	FR 3
68	Gly	Gly	Gly	Gly	Gly	Arg	
69	Thr	Thr	Thr	Thr	Thr	Thr	
70	Asp	Asp	Asp	Asp	Asp	Asp	
71	Phe	Phe	Phe	Phe	Phe	Phe	L1
72	Thr	Thr	Thr	Thr	Thr	Thr	
73	Leu	Leu	Leu	Leu	Leu	Leu	
74	Thr	Thr	Thr	Thr	Thr	Thr	
75	Ile	Ile	Ile	Ile	Ile	Ile	
76	Ser	Ser	Ser	Ser	Ser	Asn	
77	Arg	Ser	Ser	Ser	Ser	Pro	
78	Leu	Leu	Leu	Leu	Leu	Val	
79	Glu	Glu	Glu	Glu	Glu	Glu	
80	Pro	Pro	Pro	Pro	Pro	Ala	
81	Glu	Glu	Glu	Glu	Glu	Asp	

FIGURE 2 2/3

## 60.3 Light Chain Sequences

Kabat #	hVdIV Jk	h60.3 template	h60.3 Phase IV	h60.3 Phase I/II	h60.3 Phase I	m60.3
82	Asp	Asp	Asp	Asp	Asp	Asp
83	Phe	Phe	Phe	Phe	Phe	Val
84	Ala	Ala	Ala	Ala	Ala	Ala
85	Val	Val	Val	Val	Val	Thr
86	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
87	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
88	Cys	Cys	Cys	Cys	Cys	Cys
89		Gln	Gln	Gln	Gln	Gln
90		Gln	Gln	Gln	Gln	Gln
91		Arg	Ser	Ser	Ser	Ser
92		Ser	Asn	Asn	Asn	Asn
93		Asn	Glu	Glu	Glu	Glu
94		Trp	Asp	Asp	Asp	Asp
95		Pro	Pro	Pro	Pro	Pro
96	Trp, Tyr, Phe, Leu, Ile		Arg	Arg	Arg	Arg
97	Thr		Thr	Thr	Thr	Thr
98	Phe		Phe	Phe	Phe	Phe
99	Gly		Gly	Gly	Gly	Gly
100	Gln, Pro, Gly		Gly	Gly	Gly	Gly
101	Gly		Gly	Gly	Gly	Gly
102	Thr		Thr	Thr	Thr	Thr
103	Lys, Arg		Lys	Lys	Lys	Lys
104	Val, Leu		Leu	Leu	Leu	Leu
105	Glu, Asp		Glu	Glu	Glu	Glu
106	Ile		Ile	Ile	Ile	Ile
107	Lys		Lys	Lys	Lys	Lys
108	Arg		Arg	Arg	Arg	Arg

\* The h60.3 light chain was made with the sequence shown in the Phase I/II column, with the following exceptions:

L68: Arg instead of Gly  
 L75: Tyr instead of Ile  
 L104: Val instead of Leu  
 L106: Glu instead of Ile

FIGURE 3

Oligonucleotide #1:

TTTTTTGAATTCAAGCTTTCCTGACTACATGAGTGCATTTCTGTTTTATTTCCAAT  
TTCAGATACCAACCGGAGAAATTGTGCTAACACAA

Oligonucleotide #2

AATTTCTCCGGTGGTATCTGAAATTGGAAATAAAACAGAAATGCACTCATGTAG  
TCAGGAAAGCTTGAATTCAAAAAA

Oligonucleotide #3

TCTCCAGCTACATTGTCTTTGTCTCCAGGTGAGAGAGCCACTCTATCCTGCAGAG  
CCAGTGAAAGTGTTGATAGTTATGGCAATAGT

Oligonucleotide #4

ACTATCAACACTTTCACTGGCTCTGCAGGATAGAGTGGCTCTCTCACCTGGAGAC  
AAAGACAATGTAGCTGGAGATTGTGTTAGCAC

Oligonucleotide #5

TTTATGCACTGGTACCAGCAGAAACCAGGACAGGCACCAAGGCTCCTCATCTAT  
CGTGCATCCAACCTAGAAACTGGTATCCCTGCC

Oligonucleotide #6

AGTTTCTAGGTTGGATGCACGATAGATGAGGAGCCTTGGTGCCTGTCCTGGTTTC  
TGCTGGTACCAGTGCATAAACTATTGCCATA

Oligonucleotide #7

AGGTTCAGTGGCAGTGGTTCTAGGACAGACTTCACTCTCACCTATTCTTCTCTAG  
AGCCTGAAGATTTTGCAGTGTATTACTGTACAG

**Oligonucleotide #8**

**CACTGCAAAATCTTCAGGCTCTAGAGAAGAATAGGTGAGAGTGAAGTCTGTCCT  
AGAACCACTGCCACTGAACCTGGCAGGGATAACC**

**Oligonucleotide #9**

**CAAAGTAATGAGGATCCTCGGACGTTTCGGTGGAGGCACCAAGGTGGAAGAGAA  
ACGTAAGTGCACTTTCCTCGAGGTCGACTTTTTT**

**Oligonucleotide #10**

**AAAAAAGTCGACCTCGAGGAAAGTGCACTTACGTTTCTCTTCCACCTTGGTGCCT  
CCACCGAACGTCCGAGGATCCTCATTACTTTGCTGACAGTAATA**

FIGURE 4

Oligonucleotide # 1

AAAAAAGAATTCGAGCTCTTCTGATAACGCTGTCCTTCTGTTTGCAGGTGTCCAG  
TGTCAGGTCCAACCTTGTCCAGTCCGG

Oligonucleotide #2

TTAACTTCGGCACCGGACTGGACAAGTTGGACCTGACACTGGACACCTGCAAAC  
AGAAGGACAGCGTTATCAGAAGAGCTCGAATTCTTTTTT

Oligonucleotide #3

TGCCGAAGTTAAGAAGCCTGGCGCTTCTGTGAAGGTCTCCTGCAAGGCTTCTGG  
CTACACCTTCACCGACTACTGGATGAACTGGGTTTCG

Oligonucleotide #4

CCAGGTGCCTGTCTGAACCCAGTTCATCCAGTAGTCGGTGAAGGTGTAGCCAGAA  
GCCTTGCAGGAGACCTTCACAGAAGCGCCAGGCTTC

Oligonucleotide #5

ACAGGCACCTGGACAGGGCCTAGAGTGGATGGGAAGGATTGATCCTTCCGATAG  
TGAAACTCACTACCATCAGAAGTTCCAGGGTAGGGT

Oligonucleotide #6

CGGGTCATTGTTACCCTACCCTGGAACTTCTGATTGTAGTGAGTTTCACTATCGG  
AAGGATCAATCCTTCCCATCCACTCTAGGCCCTGT

Oligonucleotide #7

AACAATGACCCGAGACACATCCACCAGCACAGTCTACATGGAACTCAGCAGCCT  
GCGATCTGAGGACACCGCAGTCTATTACTGTGCACG



**Oligonucleotide #8**

**AGCCGTCCACCTCGTGACAGTAATAGACTGCGGCGTCCTCAGATCGCAGGCTG  
CTGAGTTCCATGTAGACTGTGCTGGTGGATGTGTCT**

**Oligonucleotide #9**

**AGGTGGACGGCTCGGTTCTTTGCTATGGACTACTGGGGTCAAGGCACCCTCGTC  
ACCGTCTCCTCAGGTGATGCCTCACACTCGAGGTCGACTTTTTT**

**Oligonucleotide #10**

**AAAAAAGTCGACCTCGAGTGTGAGGACTCACCTGAGGAGACGGTGACGAGGGT  
GCCTTGACCCCAAGTAGTCCATAGCAAAGGAACCG**

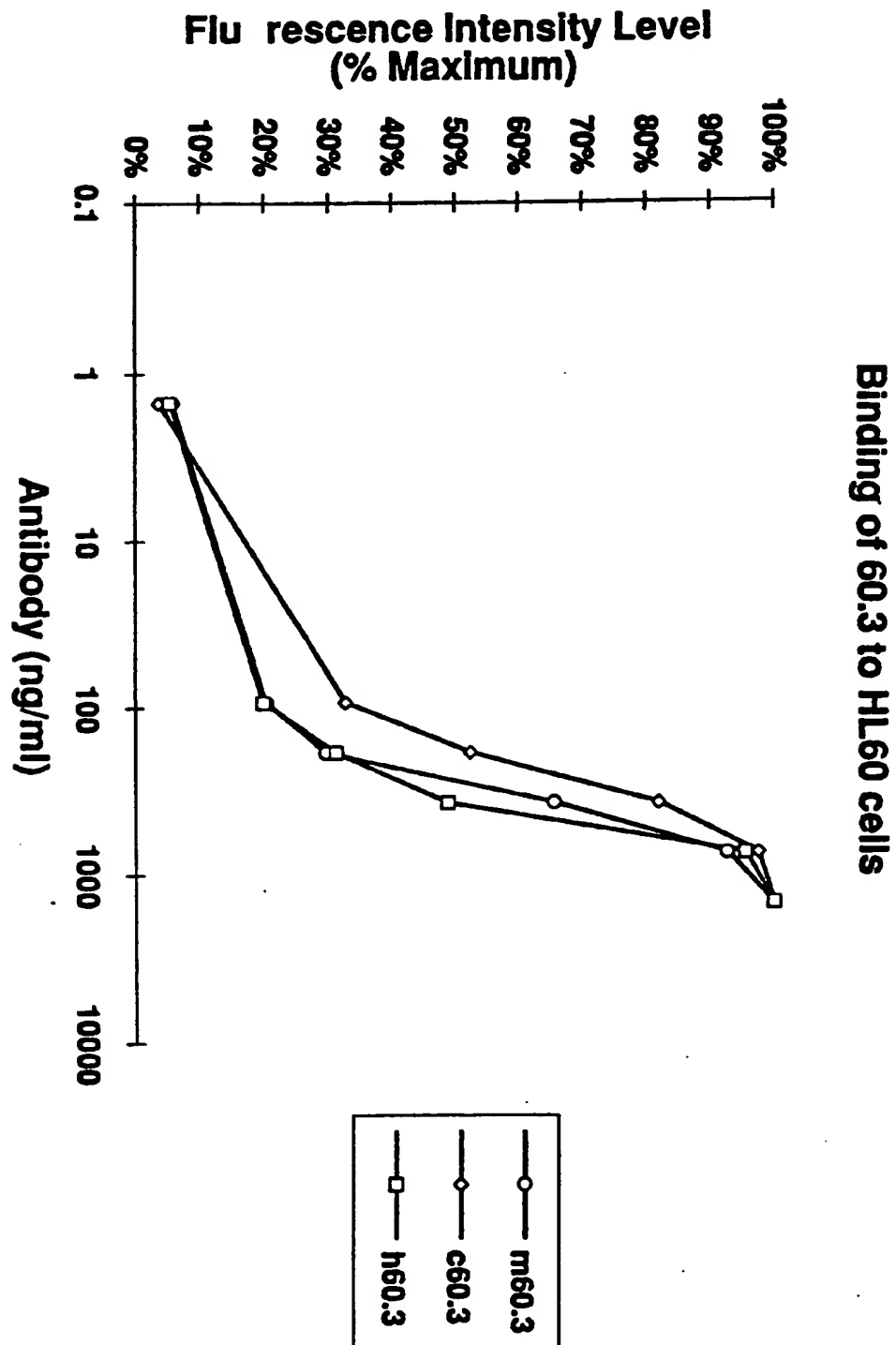


FIGURE 5

**FACS Binding Assay**  
**Preincubation with chimeric or humanized 60.3**

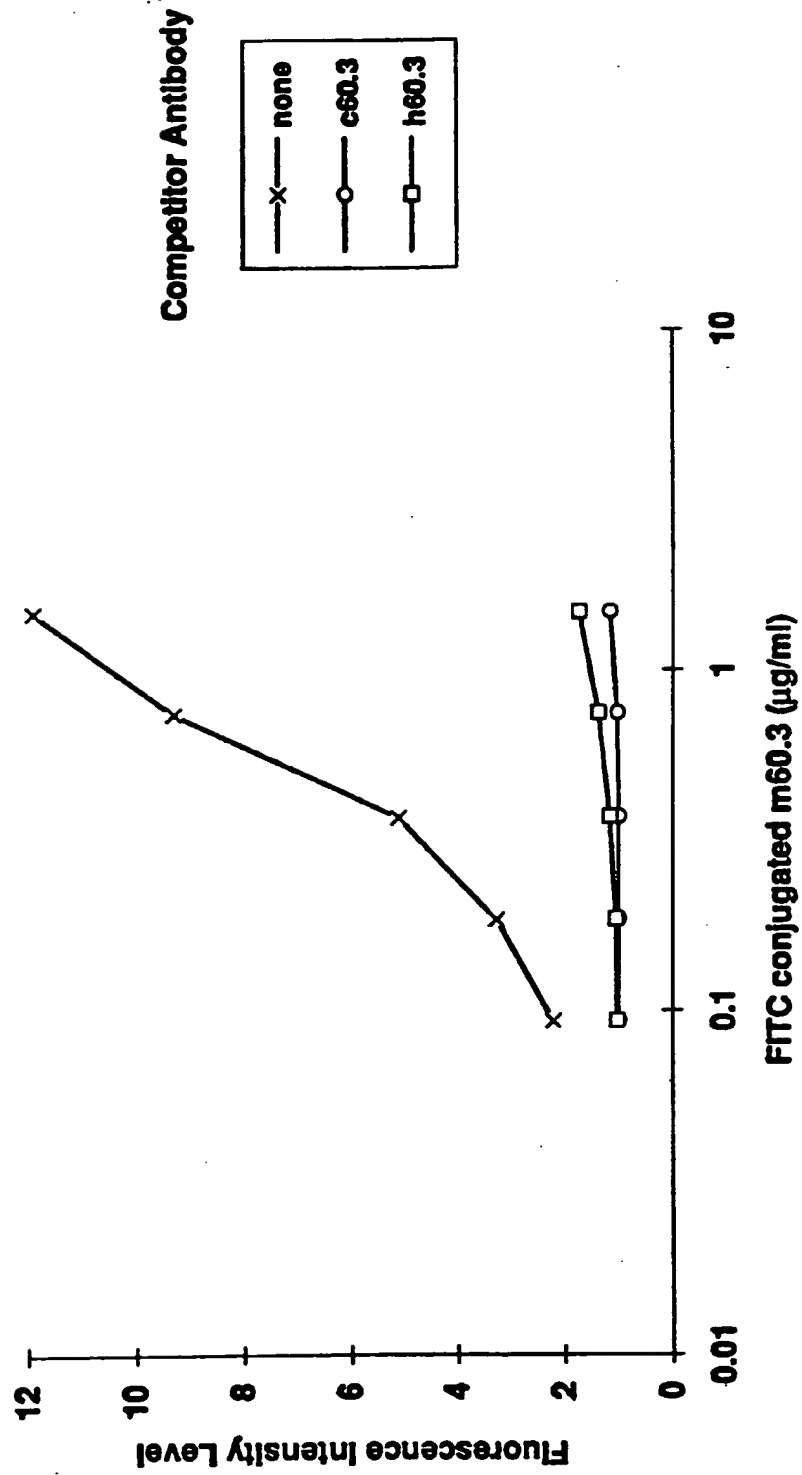


FIGURE 6

# **FACS Analysis** **Binding of FITC-m60.3 to HL60 cells**

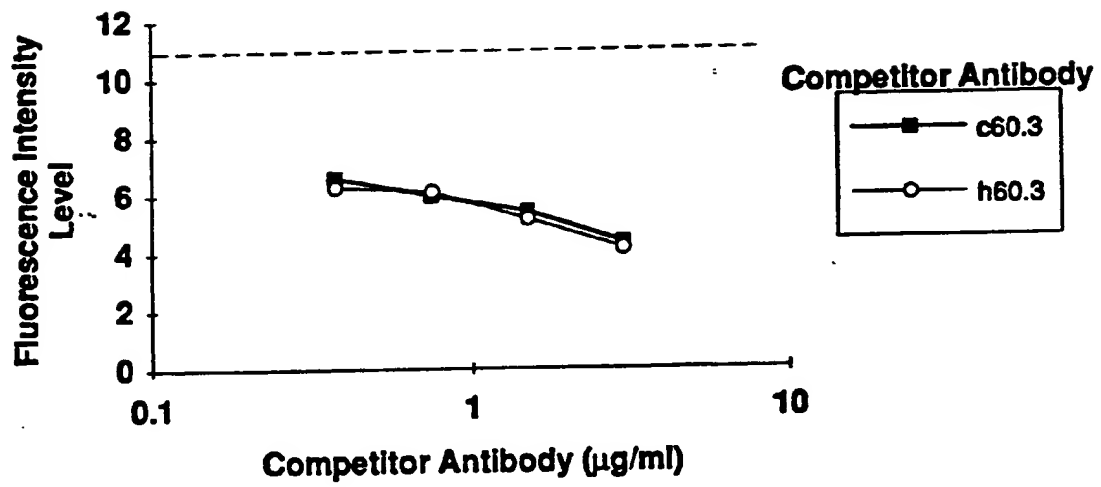


FIGURE 7

Chemiluminescence Assay  
Binding of mAbs 60.3 to Human Neutrophils

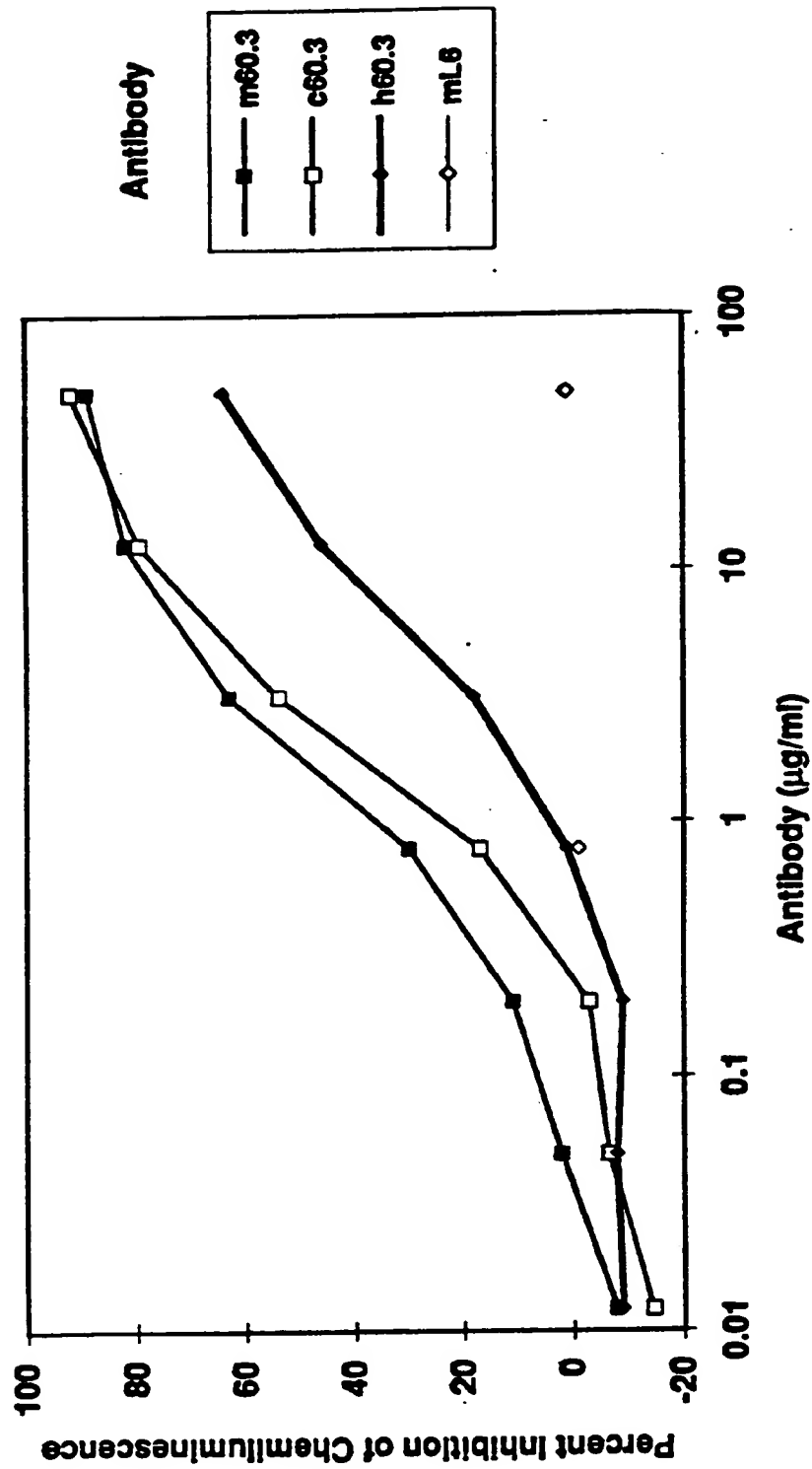


FIGURE 8

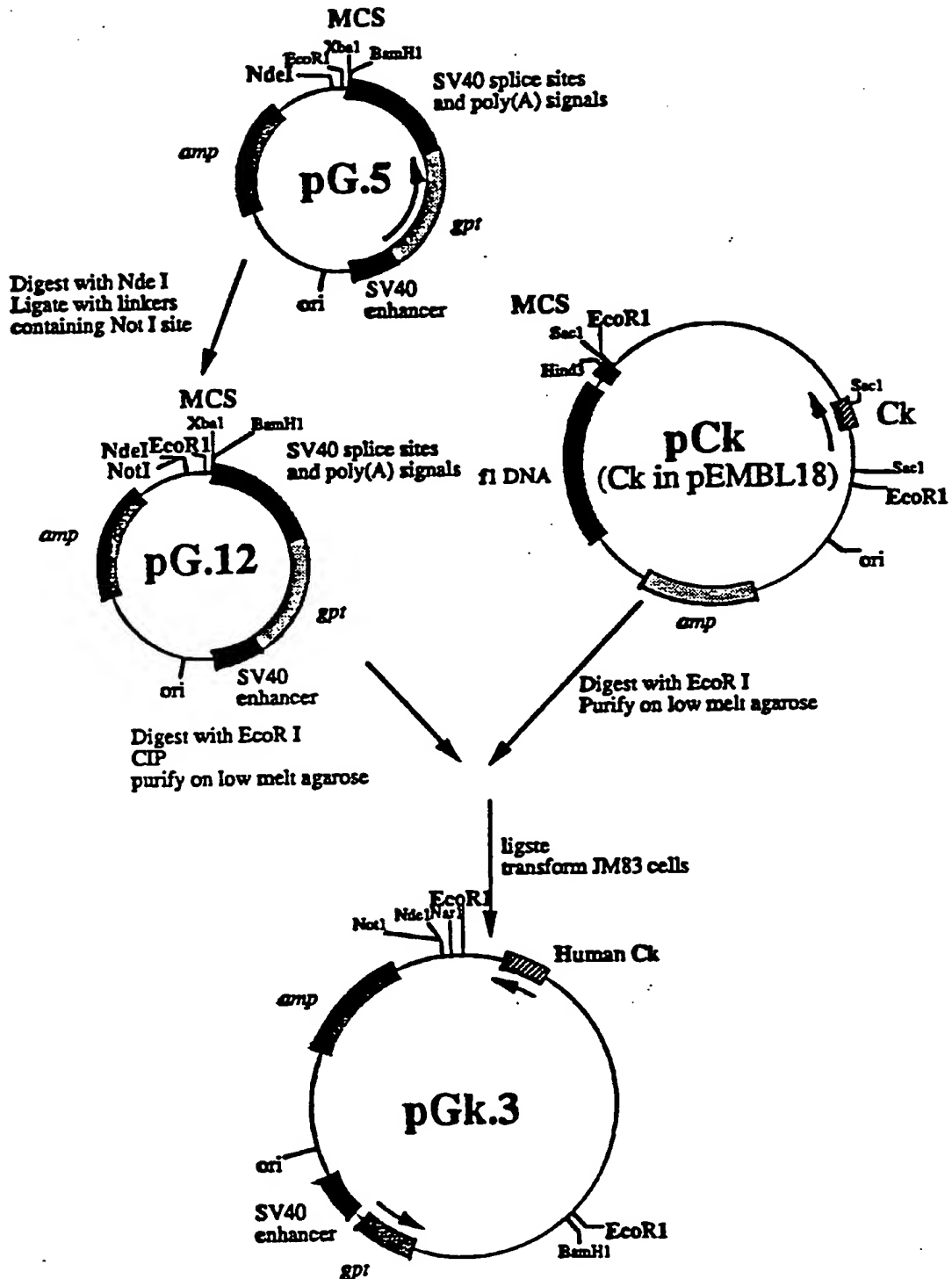


FIGURE 9 1/4

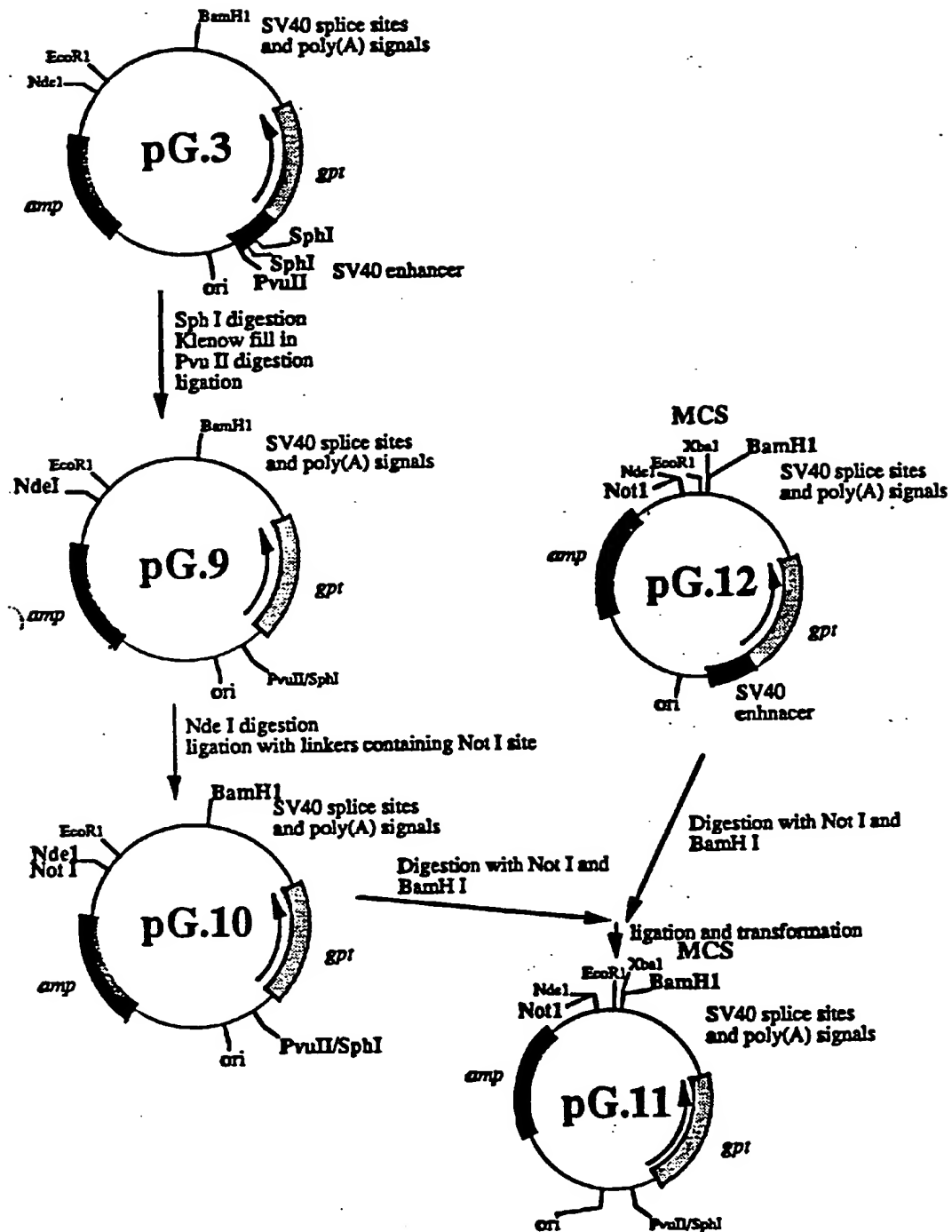


FIGURE 9 2/4

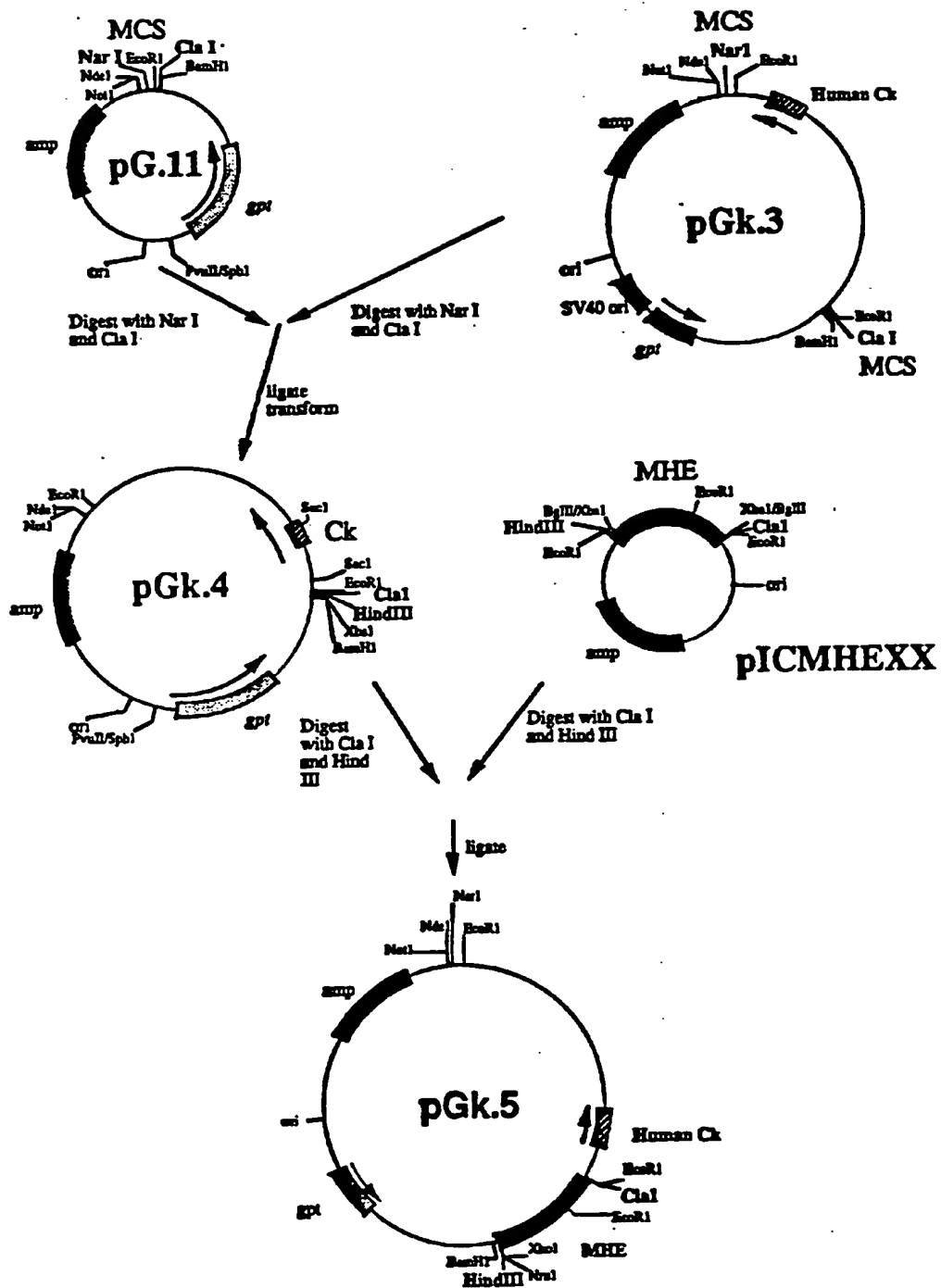


FIGURE 9 3/4



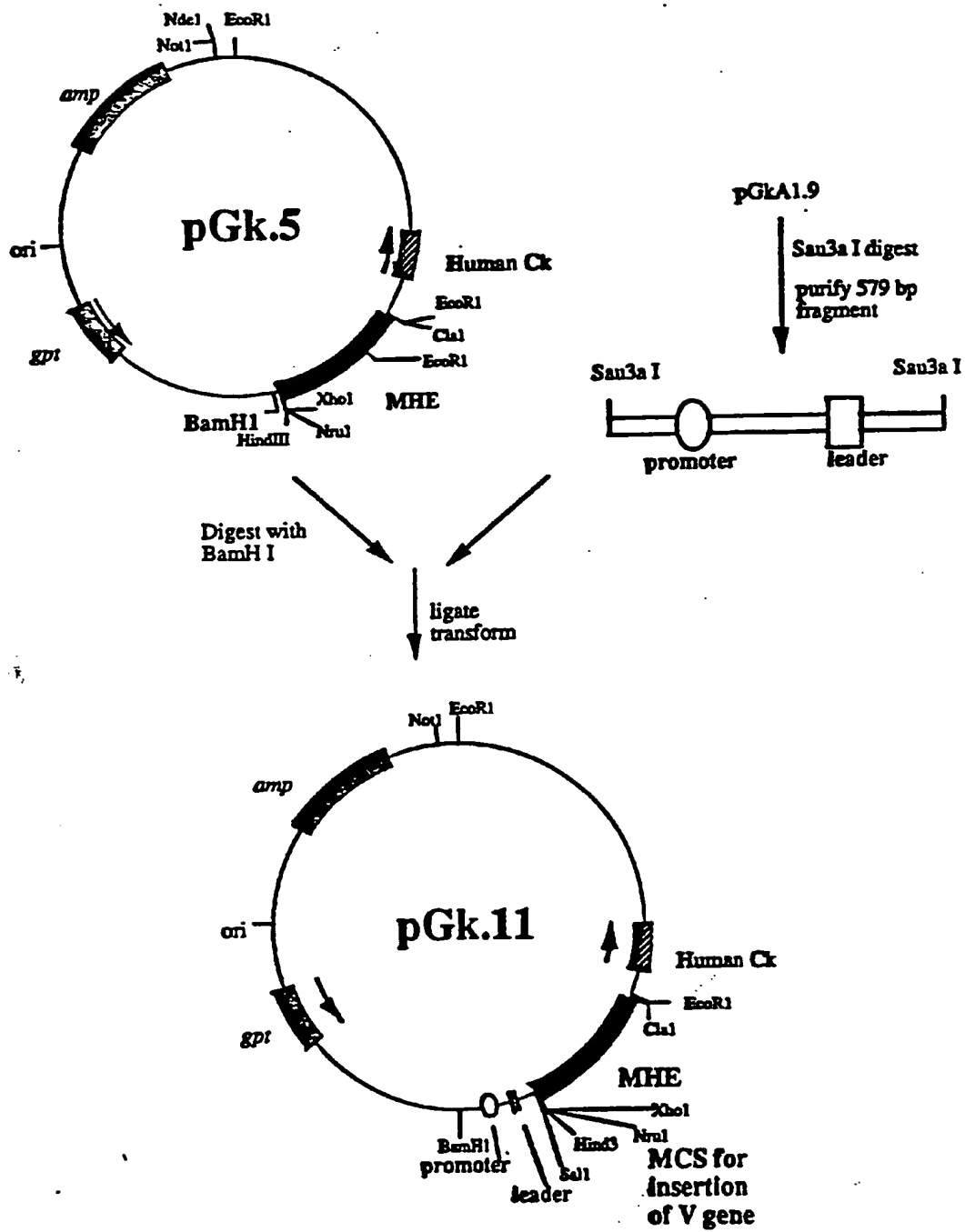


FIGURE 9 4/4

## pGk.11

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101 -----
151 -----
201 -----
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301 -----
351 -----
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501 -----
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601 -----
651 -----
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1301 -----
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1401 -----
1451 -----
1501 -----
1551 -----
1601 -----
1651 -----
1701 -----GGCC CAGGGGACTG TGAGGACAGA AGGCTTGTGG GTTTGAGGGA
1751 GGAAGTCTTT GCAGAGGATG ATAGGGTAAA ATAGAATGAA GGATGATTTT
1801 TATAAATGGT TACGTGCCTT AGGATGACTA CATATTTAGT CCCTTATAAG
1851 AGAAATTGAG TAGTTGGTAA AACAAACAGAT AATAATTATT AAATGAGGAA
1901 AGAGAGAAAC CACAGGTGCA AAGATTCAC TTTATTTATTC ATTCTCCTCC
1951 AACATTAGCA TAATTAAAGC CAAGGAGGAG GAGGGGGGTG AGGTGAAAGA
2001 TGAGCTGGAG GACCGCAATA GGGGTAGGTC CCCTGTGGAA AAAGGGTCAG
2051 AGGCCAAAGG ATGGGAGGGG GTCAGGCTGG AACTGAGGAG CAGGTGGGGG
2101 CACTTCTCCC TCTAACACTC TCCCCTGTG AAGCTCTTTG TGACGGGCGA
2151 GCTCAGGCCC TGATGGGTGA CTTCGCAGGC GTAGACTTTG TGTTCCTCGT
2201 AGTCTGCTTT GCTCAGCGTC AGGGTGCTGC TGAGGCTGTA GGTGCTGTCC
2251 TTGCTCTCCT GCTCTGTGAC ACTCTCCTGG GAGTTACCCG ATTGGAGGGC
2301 GTTATCCACC TTCCACTGTA CTTTGGCCTC TCTGGGATAG AAGTTATTCA
2351 GCAGGCACAC AACAGAGGCA GTTCCAGATT TCAACTGCTC ATCAGATGGC
2401 GGAAGATGA AGACAGATGG TGCAGCCACA GTTCCTGAGG AAAGAAGCAA
2451 ACAGGATGGT GTTTAAGTAA CAAAGTTCTG CCCTTGGGTG TGTGTTTGC
2501 GGATAAGGGC ATGTTAGGGA CAGACAGAAA ACAGCATGCT TATCCAGAT
2551 AATTATAGCA AGGAGACCAA GAAGCGTATT TAAAATCTTG ATGTTTTGAG
2601 TTTCTTCCTA GCTTCCCCCT ATTCCCTAAT AAAGTTCTAA ATTGTTTTGT
2651 TGGAGCTCTT TGCAGCCATT CTGAGGGCTT TGCATGCTTT TCTGACCTTG
2701 CAGTAAACTC AATGCTTTAG GCAAAGAATG GCCACGTCAT CCGACCCCTT
2751 CAGAGTTTAG AATTCATCGA TATCTAGATC CTAGATAATT GCATTCATTT

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FIGURE 10 1/4

## pGk.11

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2851 AAGATTTGTG AAGCCGTTTT GACCAGAATG TCAAAGTCTT AATAGTAAGG
2901 CAAAACAAAC AACTAAAAAA GATCATGAAC AAAGTCACTG TAAAGACTTC
2951 GGGTATTGGA AAATAATTGA ATGGAGACCA ATAATCAGAG GGAAGAATAA
3001 TAGAGTAATT TTAAGAAGTT TTCTAAATAT ATTAGAAATT AAAGACACTA
3051 AAGTCCTTCA ATTTCTTACA TAACCTAATT TTGAAAATGA ATTCTAAATA
3101 CATTTTAGAA GTCGATAAAC TTAAGTTTGG GGAAACTAGA ACTACTCAAG
3151 CTAAAATTAA AAGGTTGAAC TCAATAAGTT AAAAGAGGAC CTCTCCAGTT
3201 TCGGCTGAAT CCTCAACTTA TTTTAGAAAT GCAAATTACC CAGGTGGTGT
3251 TTTGCTCAGC CTGGACTTTC GGTTTGGTGG GGCTGGACAG AGTGTTCCAA
3301 AACCACTTCT TCAAACCACA GCTACAAGTT TACCTAGTGG TTTTATTTTC
3351 CCTTCCCCAA ATAGCCTTGC CACATGACCT GCTTCTTGCC AGCTGCTGCA
3401 GGTGTTCTGG TTCTGATCGG CCATCTTGAC TCAACTCAAC ATTGCTCAAT
3451 TCATTTAAAA ATATTTTAAA CTTAATTTAT TATTGTTAAA AGTCAGTTCT
3501 GGATAGGGTA TGAGAGAGCC TCACTCCCAT TCCTCGGTTA AACTTTAAGT
3551 AATGTCAGTT CTACACAAC AAGACCTCAA ATTGATTGAC AAAAATTTTG
3601 GACATTTAAA AAAATGAGTA CTTGAAAACC CTCTCACATT TTAAAGTCAC
3651 AGTATTTAAC TATTTTTCCT AGGAACCAAC TTAAGAGTAA AAGCAACATC
3701 TTCTAATATT CCATACACAT ACTTCTGTGT TCCTTTGAAA GCTGGACTTT
3751 TGCAGGCTCC ACCAGACCTC TCTAGGATCT CGAGCTCGCG AAAGCTTGCA
3801 TGCCTGCAGG TCGACTCTAG AGGATCAAAC CAACTGTCTT TGAGTAGAGC
3851 CAAAATTGTT GATATACTTT GAATTTTAAAT TATATTTCTT GCTGAGCAGA
3901 GGTGGCAAGA GTTTTCACTA ATGTGCAAAA CCACCTCATG TTCCCCTCAC
3951 CTGGGAGCCA GAGTAGCAGG AGGAAGAGAA GCTGAGCTGG GGCTTCCATG
4001 GTTCCCTCTG GGTCCTAACT GAGCAGTTCC TCCCAGGGC TCTGACACAG
4051 GCATTGATAT GGGCTCTGGA AGGTAGGGCA GCTGGGAGGG ACATGCAAAG
4101 CAGCTGGGTG GGAGCTGAGC TTCCAGCTGC AGAGACCACC TGCTTCTTCC
4151 TCTCTGCACT GAGCATCCTG CGCCACCCTG GTTGTGAGGC CAGAAAAGTC
4201 TGTGGGCTCA GTCTGAGTGT AGAACTTCTC CCTTGTGCTC AGAGAATTTT
4251 ATTCCATATG CTTTCTTCTC CTCATCACC TAAATTCACC CAGATGATGT
4301 TTGGCACAAG CCTGTTAAGA ACAATATAAA AGGCTGTGTT TTCATTTCTC
4351 TCTTCCTATC CTCAATATGC CCAGTCATCT CCCTAAGTGC ATTATTGGAT
4401 CGAGCATGA TAAGATACAT TGATGAGTTT GGACAAACCA CAACTAGAAT
4451 GCAGTGAAAA AAATGCTTTA TTTGTGAAAT TTGTGATGCT ATTGCTTTAT
4501 TTGTAACCAT TATAAGCTGC AATAAACAAG TTAACAACAA CAATTGCATT
4551 CATTTTATGT TTCAGGTTCA GGGGGAGGTG TGGGAGGTTT TTAAAGCAA
4601 GTAAAACCTC TACAAATGTG GTATGGCTGA TTATGATCTC TAGTCAAGGC
4651 ACTATACATC AAATATTCTT TATTAACCC TTTACAAATT AAAAAGCTAA
4701 AGGTACACAA TTTTGTAGCA TAGTTATTAA TAGCAGACAC TCTATGCCTG
4751 TGTGGAGTAA GAAAAACAG TATGTTATGA TTATAACTGT TATGCCTACT
4801 TATAAAGGTT ACAGAATATT TTTCCATAAT TTTCTTGAT AGCAGTGCAG
4851 CTTTTTCCTT TGTGGTGTAA ATAGCAAAGC AAGCAAGAGT TCTATTACTA
4901 AACACAGCAT GACTCAAAAA ACTTAGCAAT TCTGAAGGAA AGTCCTTGGG
4951 GTCTTCTACC TTTCTCTTCT TTTTGGAGG AGTAGAATGT TGAGAGTCAG
5001 CAGTAGCCTC ATCATCACTA GATGGCATT CTCTGAGCA AAACAGGTTT
5051 TCCTCATTAA AGGCATTCCA CCACTGCTCC CATTATCAG TTCCATAGGT
5101 TGGAATCTAA AATACACAAA CAATTAGAA CAGTAGTTA ACACATTATA
5151 CACTTAAAAA TTTTATATTT ACCTTAGAGC TTTAAATCTC TGTAGGTAGT
5201 TTGTCCAATT ATGTCACACC ACAGAAGTAA GGTTCCTTCA CAAAGATCCG
5251 GGGCCCACTC ATAAATCCAG TTGCCGCCAC GGTAGCCAAT CACCGTATCG
5301 TATAAATCAT CGTCGGTACG TTCGGCATCG CTCATCACAA TACGTGCCTG
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5451 TGCACAGGCG GTTGTGCGCC GTGATTAAAG CGGCGGACTA GCGTCGAGGT
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5551 GTGTCATGGA TGCAGCCTCC AGAATACTTA CTGGAACTA TTGTAACCCG

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FIGURE 10 2/4

## pGk.11

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2851 AAGATTTGTG AAGCCGTTTT GACCAGAATG TCAAAGTCTT AATAGTAAGG
2901 CAAAACAAAC AACTAAAAAA GATCATGAAC AAAGTCACTG TAAAGACTTC
2951 GGGTATTGGA AAATAATTGA ATGGAGACCA ATAATCAGAG GGAAGAATAA
3001 TAGAGTAATT TTAAGAAGTT TTCTAAATAT ATTAGAAATT AAAGACACTA
3051 AAGTCCTTCA ATTTCTTACA TAACCTAATT TTGAAAATGA ATTCTAAATA
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3151 CTAAATTAAT AAGGTTGAAC TCAATAAGTT AAAAGAGGAC CTCTCCAGTT
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3401 GGTGTTCTGG TTCTGATCGG CCATCTTGAC TCAACTCAAC ATTGCTCAAT
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3751 TGCAGGCTCC ACCAGACCTC TCTAGGATCT CGAGCTCGCG AAAGCTTGCA
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4001 GTTCCCTCTG GGTCTTAACCT GAGCAGTTCC TCCCAGGGG TCTGACACAG
4051 GCATTGATAT GGGCTCTGGA AGGTAGGGCA GCTGGGAGGG ACATGCAAG
4101 CAGCTGGGTG GGAGCTGAGC TTCCAGCTGC AGAGACCACC TGCTTCTTCC
4151 TCTCTGCACT GAGCATCCTG CGCCACCCTG GTTGTCAGGC CAGAAAAGTC
4201 TGTGAGTCA GTCTGAGTGT AGAAGTTCTC CTTGTGCTC AGAGAATTTT
4251 ATTCCATATG CTTTCTTCTC CTCAATCACC TAAATTCACC CAGATGATGT
4301 TTGGCACAAG CCTGTTAAGA ACAATATAAA AGGCTGTGTT TTCATTTCTC
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5301 TATAAATCAT CGTCGGTACG TTCGGCATCG CTCATCACAA TACGTGCCTG
5351 GACGTCGAGG ATTTGCGGTG GGTCAATGCC GCGCCAGATC CACATCAGAC
5401 GGTTAATCAT GCGATACCAG TGAGGGATGG TTTTACCATC AAGGGCCGAC
5451 TGCACAGGCG GTTGTGCGCC GTGATTAAAG CGGCGGACTA GCGTCGAGGT
5501 TTCAGGATGT TTAAGCGGG GTTTGAACAG GGTTCGCTC AGGTTTGCCT
5551 GTGTCATGGA TGCAGCCTCC AGAATACTTA CTGAAACTA TTGTAACCCG

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FIGURE 10 2/4

## pGk.11

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5701 TCAATCCAGG TATCTTGCGG GATATCAACA ACATAGTCAT CAACCAGCGG
5751 ACGACCAGCC GGTTTTGCGA AGATGGTGAC AAAGTGCCT TTTGGATACA
5801 TTTCACGAAT CGCAACCGCA GTACCACCGG TATCCACCAG GTCATCAATA
5851 ACGATGAAGC CTTGCGCATC GCCTTCTGCG CGTTTCAGCA CTTTAAGCTC
5901 GCGCTGGTTG TCGTGATCGT AGCTGGAAAT ACAAACGGTA TCGACATGAC
5951 GAATACCCAG TTCACGCGCC AGTAACGCAC CCGGTACCAG ACCGCCACGG
6001 CTACGGCAA TAATGCCTT CCATTGTTCA GAAGGCATCA GTCGGCTTGC
6051 GAGTTTACGT GCATGGATCT GCAACATGTC CCAGGTGACG ATGTATTTTT
6101 CGCTCATGTG AAGTGTCCCA GCCTGTTTAT CTACGGCTTA AAAAGTGTTC
6151 GAGGGGAAAA TAGGTTGCGC GAGATTATAG AGATCAGCTT TTTGCAAAAG
6201 CCTAGGCCTC CAAAAAGCC TCCTCACTAC TTCTGGAATA GCTCAGAGGC
6251 CGAGCGGCC TCGGCCTCTG CATAAATAAA AAAAATTAGT CAGCCATGGG
6301 GCGGAGAAAT GGGCGGGATG GCGCGAGTTA GGGCGGAAC TGGCGGAGTT
6351 AGGGGCGGGA CTATGGTTGC TGACTAATTG AGATGCTGCA TTAATGAATC
6401 GGCCAACGCG CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT CTTCCGCTTC
6451 CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
6501 CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC
6551 GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
6601 AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
6651 ATCACAATAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
6701 TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT
6751 TCCGACCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTGGGAA
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6901 CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC
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7051 CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCTAGGTA
7101 CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAAACA AACCACCGCT
7151 GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
7201 AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT
7251 GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
7301 ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTAA AATCAATCTA
7351 AAGTATATAT GAGTAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
7401 AGGCACCTAT CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA
7451 CTCCCCGTCG TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC
7501 CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT
7551 CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCTTGCA
7601 ACTTTATCCG CCTCCATCCA GTCTATTAAT TGTGCGGGG AAGCTAGAGT
7651 AAGTAGTTCC CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG
7701 GCATCGTGGT GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT
7751 TCCCAACGAT CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC
7801 GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG
7851 TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCTATG
7901 CCATCCGTAA GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT
7951 CTGAGAATAG TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAATAC
8001 GGGATAATAC CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTTGA
8051 AAACGTTCTT CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC
8101 CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA
8151 CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA
8201 AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT
8251 TTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT
8301 ACATATTTGA ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA
8351 TTTCCCCGAA AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC

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FIGURE 10 3/4

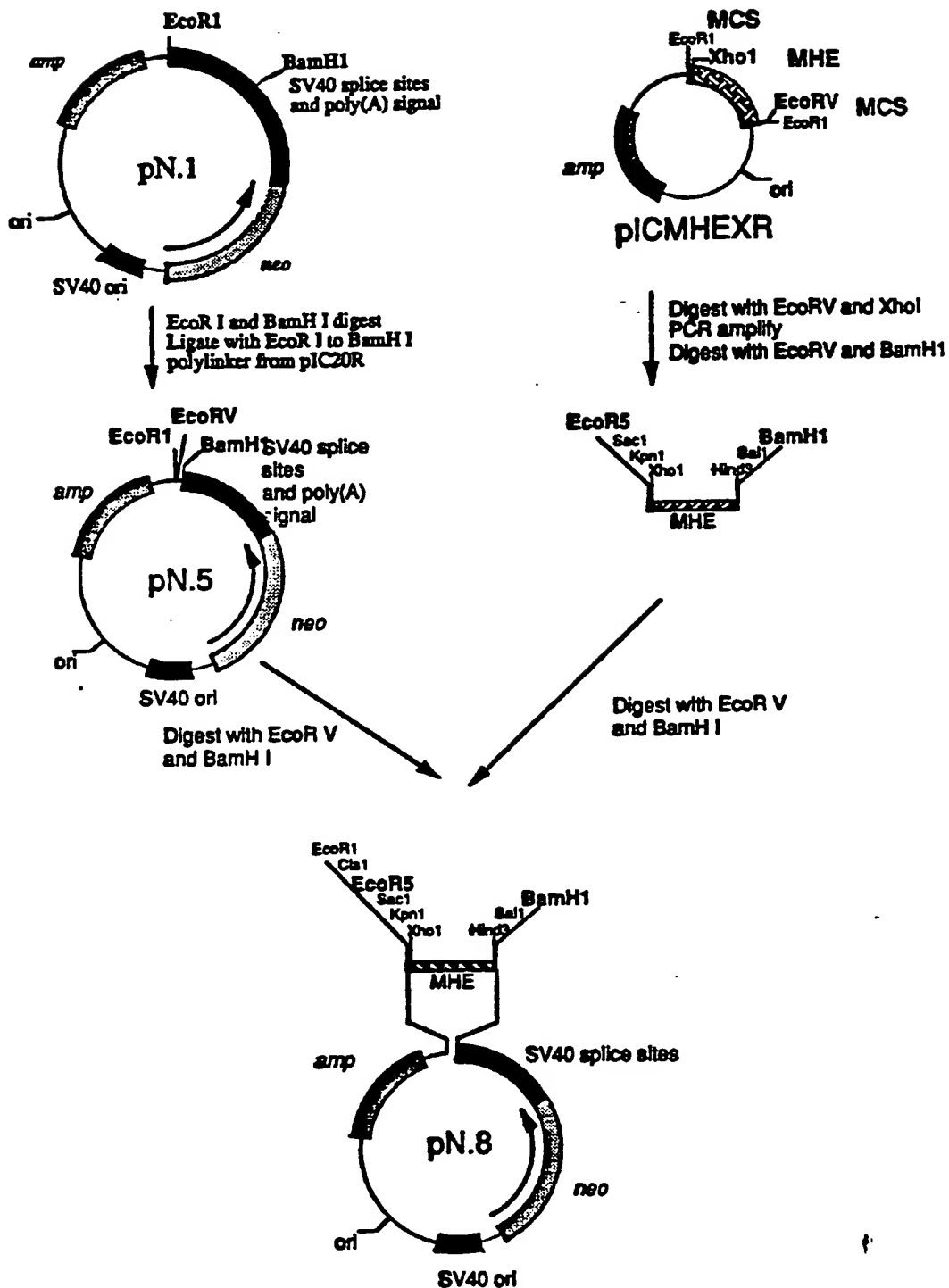


FIGURE 11 1/3

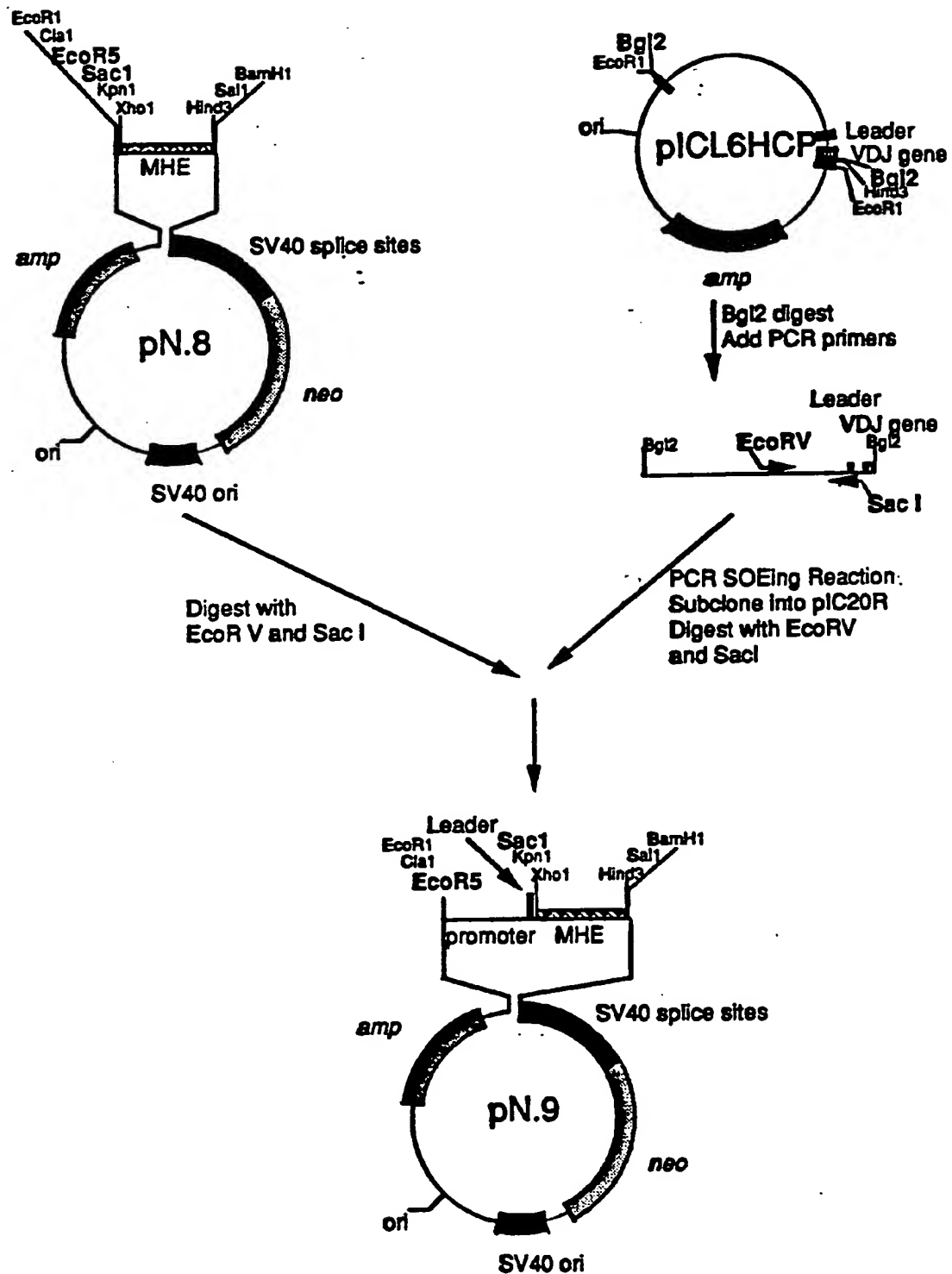


FIGURE 11 2/3

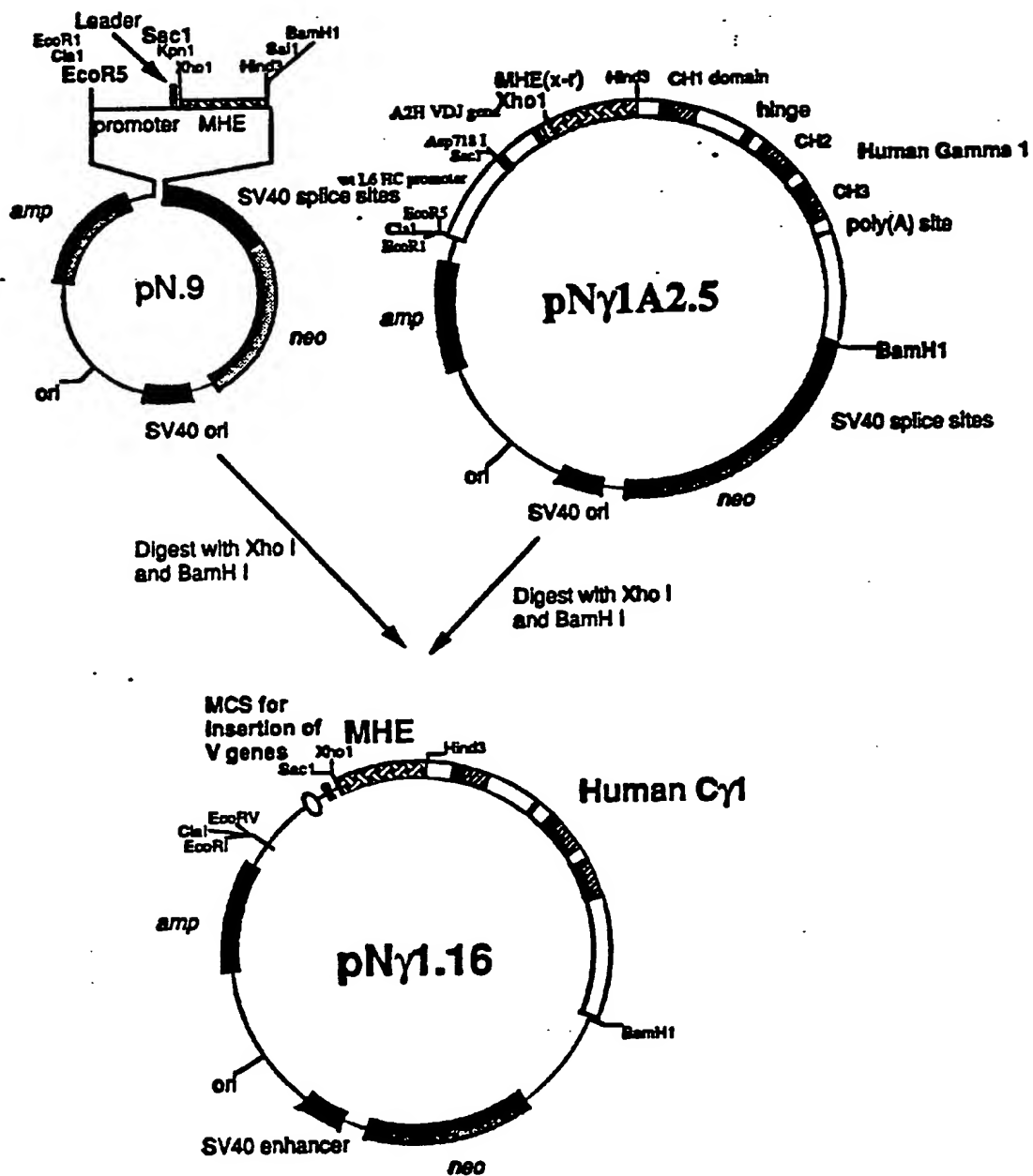


FIGURE 11 3/3



## pNg1.16(2)

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101 AGCCAGACA CTGGACGCTG AACCTCGCGG ACAGTTAAGA ACCCAGGGGC
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351 CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTTC AGGACTCTAC
401 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC
451 CTACATCTGC AACGTGAATC ACAAGCCAG CAACACCAAG GTGGACAAAC
501 GCGTTGGTGA GAGGCCAGCA CAGGGAGGGA GGGTGTCTGC TGAAGCCAG
551 GCTCAGCGCT CCTGCCTGGA CGCATCCCGG CTATGCAGCC CCAGTCCAGG
601 GCAGCAAGGC AGGCCCCGTC TGCCTCTTCA CCCGGAGGCC TCTGCCCGCC
651 CCACTCATGC TCAGGGAGAG GGTCTTCTGG CTTTTTCCCC AGGCTCTGGG
701 CAGGCACAGG CTAGGTGCCC CTAACCCAGG CCCTGCACAC AAAGGGGCG
751 GTGCTGGGCT CAGACCTGCC AAGAGCCATA TCCGGGAGGA CCCTGCCCT
801 GACCTAAGCC CACCCCAAAG GCCAACTCT CCACTCCCTC AGCTCGGACA
851 CCTTCTCTCC TCCAGATT CAGTAACTCC CAATCTTCTC TCTGCAGAGC
901 CCAAATCTTG TGACAAAAC CACACATGCC CACCGTGCCC AGGTAAGCCA
951 GCCCAGGCCT CGCCCTCCAG CTCAGGCGG GACAGGTGCC CTAGAGTAGC
1001 CTGCATCCAG GGACAGGCC CAGCCGGGTG CTGACACGTC CACCTCCATC
1051 TCTTCTCAG CACCTGAAC CTTGGGGGGA CCGTCAGTCT TCCTCTTCCC
1101 CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT
1151 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
1201 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA
1251 GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCTCACC GTCTGCACC
1301 AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAGCC
1351 TCCCAAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GTGGGACCCG
1401 TGGGGTGCGA GGGCCACATG GACAGAGGCC GGCTCGGCC ACCCTCTGCC
1451 CTGAGAGTGA CCGCTGTACC AACCTCTGTC CCTACAGGGC AGCCCCGAGA
1501 ACCACAGGTG TACACCCTGC CCCCATCTAG AGAGGAGATG ACCAAGAACC
1551 AGGTGAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
1601 GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCAAGCC
1651 TCCCGTGCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
1701 TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
1751 CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
1801 GGGTAAATGA GTGCGACGGC CGGCAAGCCC CCGTCCCCG GGCTCTCGCG
1851 GTCGCACGAG GATGCTTGGC ACGTACCCCT TGACATACT TCCCGGGCGC
1901 CCAGCATGGA AATAAAGCAC CCAGCGCTGC CCTGGGCCCC TGCGAGACTG
1951 TGATGGTTCT TTCCACGGGT CAGGCCGAGT CTGAGGCCTG AGTGGCATGA
2001 GGGAGGCAGA GCGGTCCCA CTGTCCCCAC ACTGGCCCAG GCTGTGCAGG
2051 TGTGCCTGGG CCCCCTAGGG TGGGGCTCAG CCAGGGGCTG CCCTCGGCAG
2101 GGTGGGGGAT TTGCCAGCGT GGCCCTCCCT CCAGCAGCAC CTGCCCTGGG
2151 CTGGGCCACG GGAAGCCCTA GGAGCCCTG GGGACAGACA CACAGCCCT
2201 GCTCTGTAG GAGACTGTCC TGTCTGTGA GCGCCCTGT CCTCCGACC
2251 TCCATGCCCA CTCGGGGGCA TGCCTAGTCC ATGTGCGTAG GGACAGGCC
2301 TCCCTCACC ATCTACCCC ACGGCACTAA CCCCTGGCTG CCCTGCCAG
2351 CCTCGCACCC GCATGGGGAC ACAACCGACT CCGGGGACAT GCACTCTCGG
2401 GCCCTGTGGA GGGACTGGTG CAGATGCCCC CACACACACT CAGCCAGAC
2451 CCGTTCAACA AACCCGCAC TGAGGTTGGC CGGCCACAG GCCACCAC
2501 ACACAGTGC ACGCCTCACA CACGGAGCCT CACCCGGGCG AACTGCACAG
2551 CACCCAGACC AGAGCAAGGT CCTCGCACAC GTGAACACTC CTCGGACACA
2601 GGCCCCACG AGCCCCACG GGCACCTCAA GGCCACGAG CCTCTCGGCA
2651 GCTTCTCCAC ATGCTGACCT GCTCAGACAA ACCCAGCCCT CCTCTCACA
2701 GGGTGCCCCT GCAGCCGCCA CACACACACA GGGGATCACA CACCACGTCA
2751 CGTCCCTGGC CTGGGCCAC TTCCAGTGC CGCCCTTCCC TGCAGGACGG

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FIGURE 12 1/4

## pNg1.16(2)

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2801 ATCCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC CACAAC TAGA
2851 ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG CTATTGCTTT
2901 ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC AACAAATTGCA
2951 TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT TTTTAAAGC
3001 AAGTAAACC TCTACAAATG TGGTATGGCT GATTATGATC TCTAGTCAAG
3051 GCACTATACA TCAAATATTC CTTATTAACC CCTTTACAAA TAAAAAGCT
3101 AAAGGTACAC AATTTTTGAG CATAGTTATT AATAGCAGAC ACTCTATGCC
3151 TGTGTGGAGT AAGAAAAAAC AGTATGTTAT GATTATAACT GTTATGCCTA
3201 CTTATAAAGG TTACAGAATA TTTTCCATA ATTTCTTGT ATAGCAGTGC
3251 AGCTTTTTCC TTTGTGGTGT AAATAGCAAA GCAAGCAAGA GTTCTATTAC
3301 TAAACACAGC ATGACTCAAA AAACCTTAGCA ATTCTGAAGG AAAGTCCTTG
3351 GGGTCTTCTA CCTTTCTCTT CTTTTTTGGA GGAGTAGAAT GTTGAGAGTC
3401 AGCAGTAGCC TCATCATCAC TAGATGGCAT TTCTTCTGAG CAAAACAGGT
3451 TTTCTTCATT AAAGGCATT CACCACTGCT CCCATTCATC AGTTCCATAG
3501 GTTGAATCT AAAATACACA AACAAATTAGA ATCAGTAGTT TAACACATTA
3551 TACACTTAAA AATTTTATAT TTACCTTAGA GCTTTAAATC TCTGAGGTA
3601 GTTTGTCCAA TTATGTCACA CCACAGAAGT AAGGTTCTTT CACAAAGATC
3651 CGGGACCAAA GCGGCCATCG TGCCTCCCCA CTCCTGCAGT TCGGGGGCAT
3701 GGATGCGCGG ATAGCCGCTG CTGGTTTCCT GGATGCCGAC GGATTTGCAC
3751 TGCCGGTAGA ACTCCGCGAG GTCGTCCAGC CTCAGGCAGC AGCTGAACCA
3801 ACTCGCGAGG GGATCGAGCC CGGGGTGGGC GAAGAACTCC AGCATGAGAT
3851 CCCC GCGCTG GAGGATCATC CAGCCGGCGT CCCGGAAC GATTCCGAAG
3901 CCAACCTTT CATAGAAGGC GCGGTGGAA TCGAAATCTC GTGATGGCAG
3951 GTTGGGCGTC GCTTGGTCGG TCATTTGCGA CCCAGAGTC CCGCTCAGAA
4001 GAACTCGTCA AGAAGGCGAT AGAAGGCGAT GCGCTGCGAA TCGGGAGCGG
4051 CGATACCGTA AAGCAGGAG AAGCGGTCAG CCCATTGCGC GCCAAGCTCT
4101 TCAGCAATAT CACGGGTAGC CAACGCTATG TCCTGATAGC GGTCCGCCAC
4151 ACCCAGCCGG CCACAGTCGA TGAATCCAGA AAAGCGGCCA TTTTCCACCA
4201 TGATATTTCG CAAGCAGGCA TCGCCATGGG TCACGACGAG ATCCTCGCCG
4251 TCGGGCATGC GCGCCTTGAG CCTGGCGAAC AGTTCGGCTG GCGCGAGCCC
4301 CTGATGCTCT TCGTCCAGAT CATCCTGATC GACAAGACCG GCTTCCATCC
4351 GAGTACGTGC TCGCTCGATG CGATGTTTCG CTGGTGGTC GAATGGGCAG
4401 GTAGCCGGAT CAAGCGTATG CAGCCGCCGC ATTGCATCAG CCATGATGGA
4451 TACTTTCTCG GCAGGAGCAA GGTGAGATGA CAGGAGATCC TGCCCCGGCA
4501 CTTGCCCCAA TAGCAGCCAG TCCCTTCCCG CTTCAGTGAC AACGTCGAGC
4551 ACAGCTGCGC AAGGAACGCC CGTCGTGGCC AGCCACGATA GCCGCGCTGC
4601 CTCGTCCTGC AGTTTATTCA GGGCACCGBA CAGGTCGGTC TTGACAAAAA
4651 GAACCGGGCG CCCCTGCGCT GACAGCCGGA ACACGGCGGC ATCAGAGCAG
4701 CCGATTGTCT GTTGTGCCCA GTCATAGCCG AATAGCCTCT CCACCCAAGC
4751 GGCCGGAGAA CCTGCGTGCA ATCCATCTTG TTCAATCATG CGAAACGATC
4801 CTCATCCTGT CTCTTGATCA GATCTTGATC CCCTGCGCCA TCAGATCCTT
4851 GGCGGCAAGA AAGCCATCCA GTTTACTTTG CAGGGCTTCC CAACCTTACC
4901 AGAGGGCGCC CCAGCTGGCA ATTCCGGTTC GCTTGCTGTC CATAAAACCG
4951 CCCAGTCTAG CTATCGCCAT GTAAGCCAC TGCAAGCTAC CTGCTTTCTC
5001 TTTGCGCTTG CGTTTTCCCT TGTCCAGATA GCCCAGTAGC TGACATTCAT
5051 CCGGGGTCAG CACCGTTTCT GCGGACTGGC TTTCTACGTG TTCCGCTTCC
5101 TTTAGCAGCC CTTGCGCCCT GAGTGCTTGC GGCAGCGTGA AGCTAGCTTT
5151 TTGCAAAAGC CTAGGCCTCC AAAAAAGCCT CCTCACTACT TCTGGAATAG
5201 CTCAGAGGCC GAGGCGGCCT CGGCCTCTGC ATAAATAAAA AAAATTAGTC
5251 AGCCATGGGG CGGAGAATGG GCGGGATGG GCGGAGTTAG GGCGGAAGTC
5301 GCGGAGTTA GGGGCGGGAC TATGGTTGCT GACTAATTGA GATGCATGCT
5351 TTGCATACTT CTGCTGCTG GGGAGCCTGG GGACTTTCCA CACCTGGTTG
5401 CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC TGGGGAGCCT
5451 GGGGACTTTC CACACCCTAA CTGACACACA TTCCACAGCT GCCTCGCGCG
5501 TTTGCGTGAT GACGGTGAAA ACCTCTGACA CATGCAGCTC CCGGAGACGG
5551 TCACAGCTTG TCTGTAAGCG GATGCCGGGA GCAGACAAGC CCGTCAGGGC

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FIGURE 12 2/4

## pNg1.16(2)

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5601 GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC GCAGCCATGA CCCAGTCACG
5651 TAGCGATAGC GGAGTGTATA CTGGCTTAAC TATGCGGCAT CAGAGCAGAT
5701 TGTAAGTACA GTGCACCATA TGCGGTGTGA AATACCGCAC AGATGCGTAA
5751 GGAGAAAATA CCGCATCAGG CGCTCTTCCG CTTCTCGCT CACTGACTCG
5801 CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC
5851 GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT
5901 GAGCAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAGGC CGCGTTGCTG
5951 GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG
6001 CTCAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGCGCT
6051 TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT
6101 ACCGGATACC TGTCCGCCCT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA
6151 TAGCTCACGC TGTAGGTATC TCAGTTCCGG GTAGGTCGTT CGCTCCAAGC
6201 TGGGCTGTGT GCACGAACCC CCCGTTCCAG CCGACCGCTG CGCCTTATCC
6251 GGTAACATATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT
6301 GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG
6351 CTACAGAGTT CTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA
6401 GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAGAGT
6451 TGGTAGCTCT TGATCCGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT
6501 TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
6551 CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAATCACC
6601 TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC
6651 TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA
6701 ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC
6751 GATCTGTCTA TTTCGTTTCA CCATAGTTGC CTGACTCCCC GTCGTGTAGA
6801 TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCAGTGC TGAATGATA
6851 CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC
6901 AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA
6951 TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT
7001 AATAGTTTGC GCAACGTTGT TGCCATTGCT GCAGGCATCG TGGTGTACG
7051 CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC
7101 GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT
7151 CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT
7201 TATGGCAGCA CTGCATAATT CTCCTACTGT CATGCCATCC GTAAGATGCT
7251 TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG
7301 CCGCGACCGA GTTGCTCTTG CCCGGCGTCA ACACGGGATA ATACCGCGCC
7351 ACATAGCAGA ACTTTAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC
7401 GAAAACCTCT AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC
7451 ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC
7501 TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGGG
7551 CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA
7601 AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT
7651 TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC
7701 CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT
7751 AGGCGTATCA CGAGGCCCTT TCGTCTTCAA GAATTCATCG ATATCGGAAA
7801 ATGAAAAAAA ATATTTTTTA ATTTTAAAT GAAATGTTTA TTTTCAATTT
7851 CTCCAAATTT CACAAGGAAA GATTAGTCAC GGGTATGGGA GAGCAGAGGA
7901 CCATAAGAGT TCAGGAATAG AATCCATTAT GATTCTGGAG TCAAGGAAGT
7951 ACTGATGCCA AGGTTTCAGT ATAAGAGCAG TATCCACTGG AAAGGATAAA
8001 GTCATAACAA CTGAGCACAG AGCAGGACAG CTACCTAATG AGTGGTCACT
8051 AATGGGCCAC TGTTACACTG TTATACGGCT TAGGAATGAG CACTGAGGCT
8101 GTGAGGTGTA TGGGTAAGGA CATCAGGATG TAAACCCAGC TCAGGTAGAG
8151 GACTCAGAGC ACAGCACAAT CAGCACGAAC TAATAAACAA CAGATAAGAT
8201 AAGGCACAAG CTCAGCAATA TTGGATCAGG GATCTTTGTA AATCTGACTG
8251 TGTATTCACT CTAGTTCAAT GTGACTCATG AAGCCACCC ATATGCAAA
8301 CTAGAGAAGA CTTTAGAGTA TAAATCTGAG GCTCACCTCA CATACCAGCA
8351 AGGGAGTGAC CAGCTTGTCT TAAGGCACCA CTGAGCCCAA GTCTTAGACA

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FIGURE 12 3/4

## pNg1.16(2)

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8401 TCATGGATTG GCTGTGGAAC TTGCTATTCC TGATGGCAGC TGCCCAAGGT
8451 AAGTCATCAG AAAAAAGAGT TCCAAGGGAA ATTGAAGCAG TTCCGAGCTC
8501 GGTACCCTCG AGATCCTAGA GAGGTCTGGT GGAGCCTGCA AAAGTCCAGC
8551 TTCAAAGGA ACACAGAAGT ATGTGTATGG AATATTAGAA GATGTTGCTT
8601 TTA CTCTTAA GTTGGTTCCT AGGAAAAATA GTTAAATACT GTGACTTTAA
8651 AATGTGAGAG GGTTCACAG TACTCATTTT TTTAAATGTC CAAAATTTTT
8701 GTCAATCAAT TTGAGGTCTT GTTTGTGTAG AACTGACATT ACTTAAAGTT
8751 TAACCGAGGA ATGGGAGTGA GGCTCTCTCA TACCCTATCC AGAACTGACT
8801 TTTAACAATA ATAAATTAA TTTAAAATAT TTTTAAATGA ATTGAGCAAT
8851 GTTGAGTTGA GTCAAGATGG CCGATCAGAA CCAGAACACC TGCAGCAGCT
8901 GGCAGGAAGC AGGTCATGTG GCAAGGCTAT TTGGGGAAGG GAAAATAAAA
8951 CCACTAGGTA AACTGTAGC TGTGGTTTGA AGAAGTGTTT TTGAACACT
9001 CTGTCCAGCC CCACCAACC GAAAGTCCAG GCTGAGCAAA ACACCACCTG
9051 GGTAATTTGC ATTTCTAAAA TAAGTTGAGG ATTCAGCCGA AACTGGAGAG
9101 GTCCTCTTTT AACTTATTGA GTTCAACCTT TTAATTTTAG CTTGAGTAGT
9151 TCTAGTTTCC CCAAACCTAA GTTTATCGAC TTCTAAAATG TATTTAGAAT
9201 T

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